The Degradation Of Soil Applied Nematicides Used For The Control Of The Potato Cyst Nematodes *Globodera rostochiensis* And *G. pallida*

Thesis

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The degradation of soil applied nematicides used for the control of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*

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A thesis submitted in partial fulfilment of the requirements of the Open University for the degree of Master of Philosophy

August 1999

Harper Adams University College in collaboration with H.R.I. Wellesbourne and the British Potato Council

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Statement of advance studies

During the tenure of this project, in addition to performing and reporting the experiments in this manuscript the author has also:

- Completed an M.Sc. unit in statistical procedures
- Attended weekly research seminars at Harper Adams University College
- Received training for high pressure liquid chromatography and isoelectric focusing for speciation of potato cyst nematodes
- Attended conferences with:
  The Association of Applied Biologists – Offered papers in Nematology, Linnean Society, London, England (December, 1997)

  The European Society of Nematologists - 24th Conference, Dundee, Scotland (3-9 August 1998)
- Presented a paper at The Association of Applied Biologists – Offered papers in Nematology, Linnean Society, London, England (December, 1998)

ABSTRACT

The persistence of oxamyl and the control of potato cyst nematodes (PCN), was studied at 10 sites where Vydate was applied at commercial rates. Soil and plant samples were taken at weekly intervals for 13 weeks. Oxamyl concentrations were assessed using HPLC and potato roots stained with acid fuschin to determine invasion. Oxamyl was degraded significantly faster at one site, with a complete breakdown of the chemical 21 days after application. This was correlated with warm temperatures, moist soils and a high pH of 7.0. At 4 sites PCN invasion was observed 42 days after application, however at the other sites invasion began within 21 days. No correlation between oxamyl concentrations and control was found, however a relationship to the development of the potato was observed, with earlier invasion in faster developing plants.

At 4 sites oxamyl degraded at significantly similar rates in both the laboratory and field. These sites had shorter half-lives of 14-25 days compared to 36-42 days at the other sites. This suggested that microbial degraders were able to remain sufficiently active in stored soils, however with the other sites activity was suppressed. Soils were taken from the field before oxamyl application, and 13 weeks and 6 months after application. At six sites degradation rates increased after a second treatment, however at 4 sites no significant increase in the rate of breakdown occurred.

Total aldicarb residues degraded quicker in aldicarb treated soils, however both aldicarb and oxamyl degraded at accelerated rates in oxamyl treated and untreated soils. This was related to increased hydrolyis at the higher pH of 6.1 in the untreated soil compared to 4.7 in the treated soils. Oxamyl degraded at similar rates in the aldicarb treated and untreated soils suggesting the breakdown was by abiotic transformations of the chemical rather than microbial degradation.

ACKNOWLEDGEMENT

The author of this thesis was in receipt of a studentship funded by the British Potato Council.

The author would like to thank Pat Haydock at HAUC for his supervision and guidance and Andy Jukes at HRI Wellesbourne for his useful advice. Additional acknowledgements go to Andy Wilcox for his invaluable statistical advice, Richard Bromilow and Peter Nicholls for help with the Persist modelling and Maximum Likelihood Programme, Avis Evans and Simon Woods for HPLC guidance and Ivan Grove for his assistance with the isoelectric focusing technique. Thank you also to the 10 growers who allowed the use of their fields for trial sites and to Paris Michaelides from Dupont who provided the analytical grade oxamyl for laboratory trials. A final acknowledgement goes to the predecessor of the project, Jonathon Proudman who carried out the methodology in Chapter 5 up to the extraction techniques.

The author would like to dedicate this thesis to her family for all their support and to Andy for always being there.

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Literature Review

1.1 Nematodes

Nematodes form a large phylum of animals known as *Nematoda*, which is probably second only to insects in the diversity of species it contains. In cultivated soils, species of nematode can be found which feed on bacteria, algae or fungi. Some species prey on small animals including other nematodes whilst others parasitise invertebrates or plants. The latter are known as plant parasitic nematodes (Whitehead, 1998).

Plant Parasitic Nematodes

It has been estimated that ten percent of world crop production is lost as a result of plant parasitic nematode feeding. Most species attack underground plant tissues, especially roots. Other species are predominately shoot parasites, attacking stems, leaves, flowers and seeds (Whitehead, 1998).

Cyst Nematodes

Cyst nematodes are generally the most important pests in temperate soil (Whitehead, 1998). They are characterised by the transformation of the mature female into a tough cyst. The most important species are; *Heterodera schachtii* on sugar beets, cabbage, and related crops; *H. avenae* on oats, barley and wheat; *H. glycines* on soyabeans; and *Globodera pallida* and *G. rostochiensis* on potato (Whitehead, 1998). Before isolation of the two species, the latter were collectively referred to as *H. rostochiensis* (Stone, 1973).

1.2 Potato Cyst Nematodes

Potato cyst nematodes or PCN, are the most problematic pests to potatoes in the UK causing yield losses of approximately 9 % of annual national production. The corresponding loss at market value has been estimated at £43m, based on the mean value of the crop from 1990-1995 (Haydock and Evans, 1998a).

Origin

The pest status of PCN can be attributed to evolved interactions between the nematode and the potato. Both were introduced into Europe from South America, the potato in about 1570 and the nematode nearly 300 years later (Evans and Haydock, 1990). The nematodes probably arrived through the importation of tubers, following the potato famines in the 1840s (Haydock and Evans, 1998b). A lack of native enemies would have enabled PCN to readily dominate European niches (Brodie et al., 1993).

The Life Cycle

The PCN life cycle (Figure 1.01) commences within tanned cysts found in the soil. The cysts are formed from the body of a dead female whose cuticle has hardened to protect up to 400 eggs within (Brodie *et al.*, 1993). The cysts remain dormant until early spring, when emergence in large numbers becomes stimulated, in response to hatching factors contained in host root exudate (Forrest and Perry, 1980). Within the cyst, the eggs embryonate into fully formed first-stage juveniles. These further moult into second-stage juveniles (J_2), before hatching from the eggs (Williams, 1978).

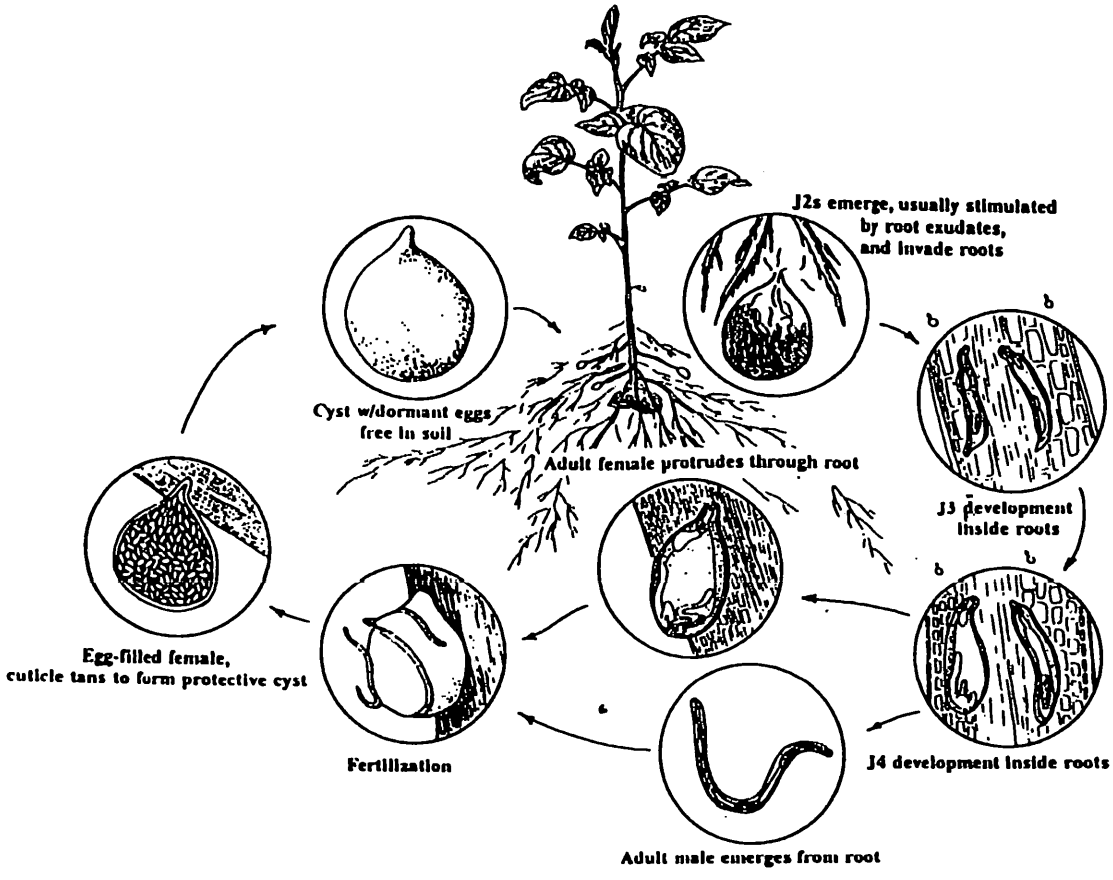


Fig. 1.01 *Life cycle of the potato cyst nematode* (after Brodie, 1984, revised by M. Brucato)

Through chemical attractions the J_2 are able to locate the potato roots, and immediately migrate through the soil towards them. They typically invade the roots behind the growing tip, or where a lateral root develops (Wyss and Grundler, 1992). Invasion is achieved using a stylet, this is similar to a sharp blade, and is used to actively probe the root cells, causing the cell walls to rupture. Once inside the roots, the J_2 continue a destructive migration through epidermal cells to the pericycle (Williams, 1978). In the pericycle a suitable cell is selected and developed into a feeding site, known as a syncytium. The J_2 secrete granules into the cell causing the dissolution of inner, and fusions of outer cells, to form a multi-nucleate feeding site. Once this

period is completed the juveniles become sedentary and are no longer capable of leaving the roots (Wyss and Grundler, 1992).

Inside the host, the juveniles are able to obtain an uninterrupted supply of food and water until they mature and die. As a result, one of the primary constraints regulating the rate of development is temperature (Jones, 1975). It has been suggested that development could be controlled more by the rate of heat accumulation than by temperatures, apart from when these are extreme. Accumulated heat is calculated in day degrees above an assumed basal temperature (Jones and Parrot, 1969). Evans (1968) studied the life cycle of *H. rostochiensis* using an assumed basal development temperature of 40 ° F (4.4 ° C) 10 cm deep, in a sandy soil. Invasion was observed to begin after 300-day degrees F (167 °C) had accumulated. After 85 days, when about 1000 day degrees F (556 °C) had accumulated, most juveniles had developed into young adults, and about 21 days later at 1500 day degrees F (833 °C) males and females were abundant (Evans, 1968).

The growing nematodes moult to third (J_3), and then to fourth-stage (J_4) juveniles. During the moult to J_4, differentiation between males and females occurs. The J_3 that developed small syncitia become J_4 vermiform males. These leave the roots and remain non-feeding in the soil for up to 10 days, where they further moult into J_5 males. The J_3 that developed a large syncitia moult to J_4 females, which continue to grow, finally moulting into adult females. The females erupt through the root surface, exposing their bodies to the external environment. The heads remain embedded in the roots enabling the females to continue feeding, whilst the eggs are produced (Brodie *et al.*, 1993). The males in the soil are attracted by stimulant pheromones secreted from the body surface of the female, and they migrate to the females and fertilise them

(Evans and Wright, 1982). Several males may surround one female resulting in multiple matings (Williams, 1978).

Upon death the bodies of the females tan to form protective cysts. These fall off the plant and remain in the soil until the next potato-growing season. The toughened tanned walls enable the cysts to withstand desiccation, and as a result, in the advent of unfavourable environmental conditions, hatching of the juveniles may slow or stop, resuming once favourable conditions return. Some cysts have been known to remain viable in the soil for up to 25 years (Baldwin and Mundo-Ocampo, 1991).

PCN species

Two PCN species exist, *Globodera rostochiensis* (Woll.) Skarbilovich and *Globodera pallida* Stone. The biology and life cycles are similar, however several morphological and behavioural differences are known to exist. Morphologically the juvenile and adult features of *G. pallida* are generally larger than *G. rostochiensis* (Stone, 1973). Isoelectric focusing (Fleming and Marks, 1983) and immunological diagnostics using PCR (Schots *et al.*, 1992) have also detected differences in the general protein and DNA patterns of the two species, respectively. Behavioural differences occur mainly during hatching.

Hatching

Whitehead *et al.* (1987) observed differences in the control of PCN populations in pot experiments where 1 litre of soil was treated with 40 mg of the nematicide, oxamyl. Oxamyl treated and untreated pots were planted with *Désirée* tubers and kept at maximum temperatures of 21 ° C. After 5 months the number of cysts and juveniles remaining in the soil

were assessed using the Fenwick can elutriation technique (Southey, 1986). Results showed that oxamyl greatly reduced the increase of *G. rostochiensis* populations, however on average it only halved the increase of *G. pallida* populations.

This failure of control of *G. pallida* could be the result of an extended period of hatching in moist soils. In pot experiments, Whitehead *et al.* (1984) found the overall hatch of *G. rostochiensis* to be rapid, ceasing after 6 weeks, whereas *G. pallida* was still emerging up to 10 weeks later. This slowness of hatch could have serious implications for the effectiveness of nematicides, such as oxamyl, which have half-lives of 2 to 3 weeks. Figure 1.02 shows how

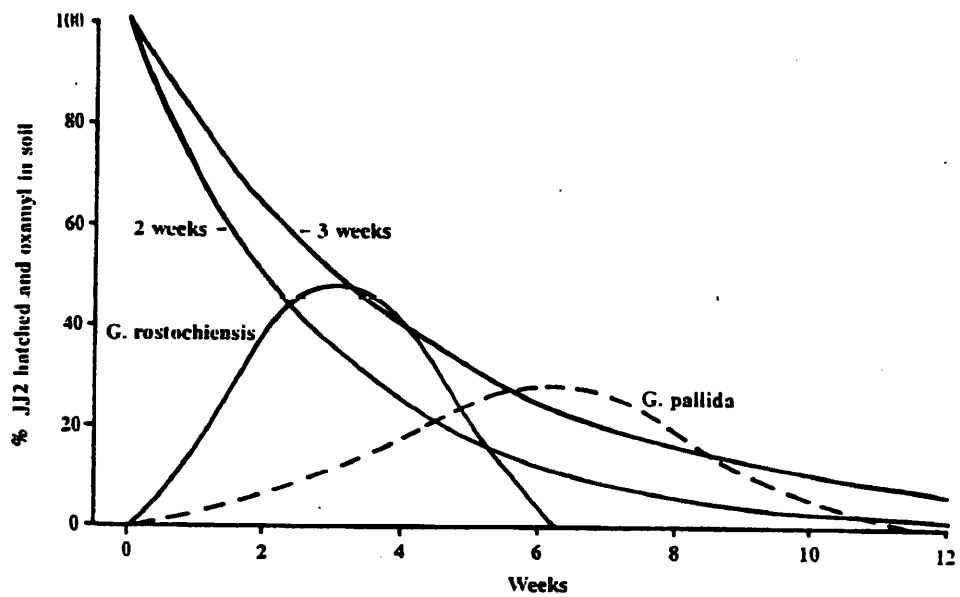


Fig. 1.02 *Hatching patterns for G. rostochiensis and G. pallida under a potato crop and decay curves for oxamyl with 2 or 3 week half-lives (Evans, 1993).*

at the slower oxamyl decay rate of 3 weeks the peak in hatching of *G. rostochiensis* is reached whilst 50 % of the nematicide still remains, compared with only 30 % at the peak of *G. pallida* hatching. At the faster decay rate, barely 10 % remains by the time that hatching of *G. pallida* reached its peak (Evans, 1993).

Lipid utilisation

Whitehead (1992) found the initial speed of emergence to differ in artificial potato root diffusate, with hatching of *G. pallida* being almost half the speed of *G. rostochiensis* over a period of six weeks. Robinson *et al.* (1987) related the rapid hatch of *G. rostochiensis* to the faster utilisation of lipid reserves during juvenile migration. At 20 °C the estimated time taken for juvenile populations to utilise 50 % of their lipid reserves was 15 days for *G. rostochiensis* and 22 days for *G. pallida.* These hatching differences could be the result of co-evolved pressures for feeding sites. The ability of *G. pallida* to store food reserves and metabolise them slowly would enable longer survival in the soil until new root growth had developed (Den Nijs and Lock, 1992).

Temperature

Franco (1979) found that *G. pallida* emerged faster at 10-20 °C whereas *G. rostochiensis* hatched more rapidly at 20-25 °C. Both species had an optimum hatch at 20 °C, however Den Nijs and Lock (1992) showed a more free emergence of *G. rostochiensis* at a range of temperatures. Again this behaviour could be a result of co-evolved interactions, as the adaptation to hatching at lower temperatures would gain *G. pallida* a competitive advantage in cooler soils.

Hatching agents

Whitehead (1992) stimulated hatching of both species in the presence of potato root diffusate (PRD), however *G. rostochiensis* juveniles also emerged in artificial agents such as sodium metavanadate and distilled water (Den Nijs and Lock, 1992). The PRD from 25 different cultivars hatched *G. rostochiensis* populations well, however some cultivars hatched *G. pallida*

well, others hatched it poorly (Evans, 1983). Artzen *et al.* (1993) also found clear differences in the hatching of *G. pallida* by 13 genotypes. This limited hatch suggested that the life cycle of *G. pallida* was synchronised with a specific host, whereas *G. rostochiensis* showed a more opportunistic behaviour (Den Nijs and Lock, 1992).

1.3 Loss to Yield

The potato

The potato, *Solanum tuberosum* is an important worldwide food commodity. In 1997, 140,000 hectares of potatoes were planted in the UK and with a mean maincrop yield of 48 t ha^{-1} approximately 7 million tonnes were produced (Haydock and Evans, 1998a). Although potato tuber quality is important, a high final yield is vital to the grower (Trudgill *et al.*, 1983).

Damage to the potato

Initial damage is caused by the destructive intracellular migration of large numbers of juveniles through epidermal cells. Light tissue damage may cause a proliferation of lateral roots, however with greater invasion the plant is eventually unable to compensate, and shows a range of symptoms resulting from a poor and inefficient root system. Infected plants are stunted and become water stressed, wilt easily, suffer nutrient shortage, become chlorotic, and eventually die prematurely, with consequent loss of yield (Evans and Haydock, 1998b). Additional stress to the plant results from the feeding of the developing nematode, and also indirectly through interactions with other organisms such as the wilt-inducing fungus *Vercillium dahliae,* which relies on the juveniles for entry into the plant (Brown, 1983).

Loss to yield

Potato cyst nematodes have one complete generation per year in the UK, the potato is their only field host, and they hatch mainly in response to specific chemicals exuded from host roots. As a result the loss to yield can be directly related to the initial population density (Pi) in the ground at planting (Trudgill *et al.*, 1996). The Pi and loss to yield are strongly density-dependent, (Figure 1.03) with high levels of invading juveniles slowing the rate of root growth and hence reducing yield (Evans and Haydock, 1990). A high competition for feeding sites one year will cause a progressively decreasing proportion of juveniles to become females, resulting in a lower Pi the following year. In contrast a low Pi will produce successful syncitia resulting in population increases of as much as 50-fold the next season (Trudgill *et al.*, 1996).

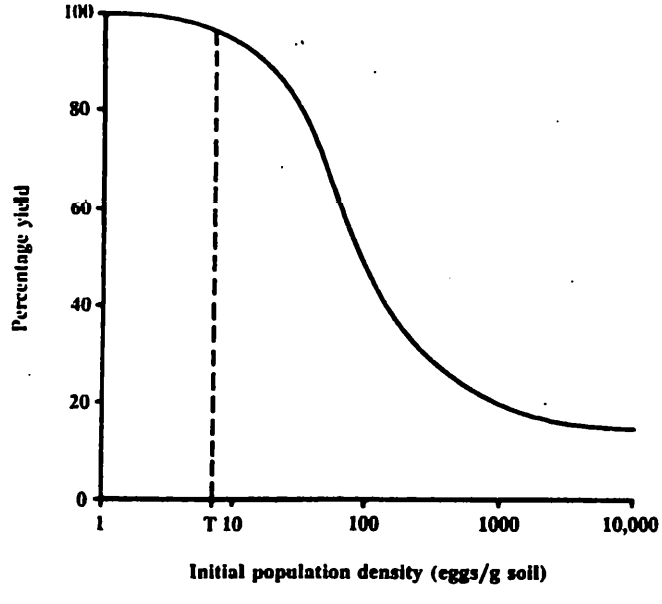


Fig. 1.03 *The relationship between yield and the number of potato cyst nematodes per gram of soil. The graph is plotted on a logarithmic scale to give a sigmoid curve. T = tolerance threshold, i.e., the nematode population density beyond which the plant cannot compensate injury* (Evans, 1993)

All plants possess a tolerance threshold, which is the point at which the host can withstand infection without suffering undue damage. When PCN are few and the Pi is below the threshold

yield is little affected as plants can compensate for any trivial injury to their roots, but as numbers increase beyond the tolerance threshold, yield begins to decline eventually reaching a minimum (Evans, 1993). Thresholds for damage vary with the potato cultivar grown and the type of soil (greatest on sandy soils), however as a general estimate yield is decreased by 2 tonnes ha $^{-1}$ for every 20 PCN eggs g $^{-1}$ soil (Trudgill *et al.*, 1996).

1.4 PCN Control

The objectives of nematode control are:

- To prevent injury to the crop plant which may result in loss of yield and quality of the produce.

- To reduce high initial populations (Pi) to a sustainable threshold density so that susceptible crops can be grown at the desired frequency (Whitehead, 1986).

With a soil infested with 100 eggs g $^{-1}$ soil and a crop plant suffering yield losses at more than 2 eggs g $^{-1}$, an immobilisation of 98 % of the Pi would be required to prevent yield losses. Lower populations of 10 eggs g $^{-1}$ would still require an 80 % immobilisation of the population to be effective (Whitehead, 1987). Methods of control include legislative control, crop rotations, the use of resistant cultivars, nematicides and cultural control (Whitehead, 1986).

Movement and monitoring

Potato cyst nematodes are spread by cultivation and harvesting machinery. Hygiene measures such as the removal of soil from machinery and the harvest of unaffected lands first can help

prevent spread. The cyst nematode is a quarantine pest within the EU so care is taken to ensure that contaminated soils are not transported across international boundaries. Council directive 69/465/EEC requires that seed potatoes for marketing are only produced on land that has been officially declared free from PCN infestation (Haydock and Evans, 1998b).

Resistant cultivars

Resistant cultivars can hinder nematode development or multiplication through a failure of the roots to produce adequate feeding sites. This results in the juveniles either dying of starvation, or moulting to males (Evans and Haydock, 1990). Theoretical differences in the final populations of *G. rostochiensis* following the harvest of resistant and susceptible cultivars are shown in Figure 1.04.

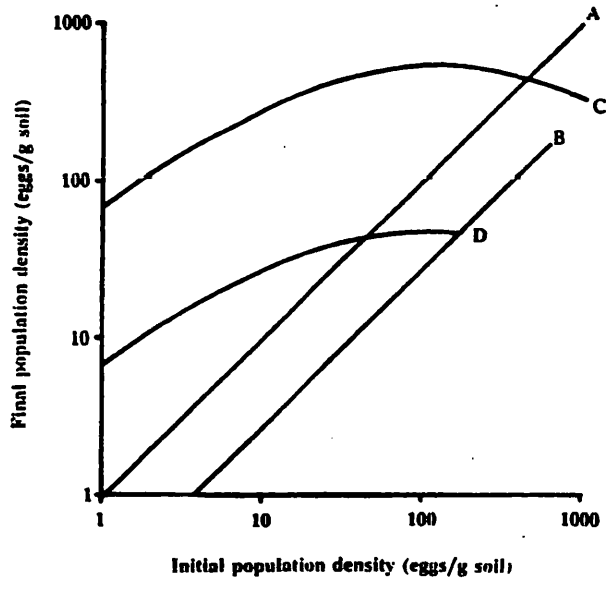


Fig. 1.04, ***Theoretical relationships between initial (Pi) and final (Pf) population densities of PCN. A) If Pf = Pi. B) If a resistant host crop is grown C) If a non-resistant potato cultivar is grown D) If a partially resistant potato cultivar is grown*** (Evans, 1993).

With the susceptible cultivar (C) the final PCN population (Pf) was similar to the initial (Pi)

resulting in no control. With the fully resistant cultivar (B) the final population was reduced by approximately two thirds (Evans, 1993).

Plant breeders have produced several resistant cultivars, the most important being Maris Piper which contains an H_1 gene providing resistance against *G. rostochiensis* Ro1 pathotypes (Evans and Haydock, 1990). The repeated growing of H_1 resistant cultivars in areas of pure *G. rostochiensis* can decrease the Pi by 80 % for each crop (Trudgill *et al.*, 1996). However cultivars with 100 % resistance to *G. pallida* have not yet been developed. Problems arise due to the polygenic virulence of *G. pallida.* A single resistance gene in the host encodes a virulent gene in *G. rostochiensis,* however a number of resistance genes are required to control the numerous virulent genes of *G. pallida* (Evans, 1993).

This lack of resistance has resulted in the selective reproduction of *G. pallida,* with small populations becoming the dominant species after 3-4 crops of an H_1 resistant cultivar (Trudgill *et al.*, 1996). Distribution maps prepared in the 1960s showed half the PCN populations in England to be essentially pure *G. rostochiensis,* however it is now estimated that no pure areas of *G. rostochiensis* remain (Evans, 1993).

Tolerant cultivars

In nematological terms the tolerance of a cultivar can be defined as the extent to which the host is able to withstand infection without suffering undue damage (Evans and Haydock, 1990). A water deficiency is the most limiting factor for yield, and so the level of tolerance possessed by a cultivar can be related to the ability of the plant to develop compensatory root growth in response to water stress (Evans and Franco, 1979).

Trudgill and Coates (1983) studied differences in the tolerance of potato cultivars by comparing the yields at several PCN field densities. Generally cultivars resistant to *G. rostochiensis* were also tolerant, for example, Maris Piper was resistant and tolerant to *G. rostochiensis*, but susceptible and intolerant of *G. pallida*. However there were exceptions as Cara which was resistant and tolerant of *G. rostochiensis*, was susceptible but outstandingly tolerant of *G. pallida* and Corsair was resistant to both, but tolerant of neither. Resistance and tolerance should be considered individually, with resistance determining the reproductive success of the nematode and tolerance the cultivar yield under nematode attack (Haydock and Evans, 1998b).

Crop rotations

Crop rotations form an effective control as potatoes are the only widely grown host crop and so populations will decrease when no host is grown (Evans, 1993). Surveys have shown decline rates to vary within and between species, (Figure 1.05), with ranges of 10 –30 % for *G. pallida*

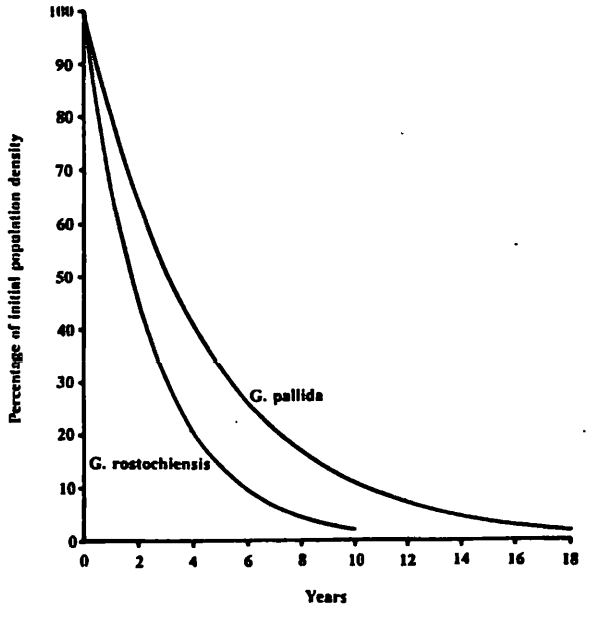


Fig. 1.05 *Decline of G. rostochiensis (33% per annum) and G. pallida (20% per annum) in the absence of host crops* (Evans, 1993)

and 20-40 % for *G. rostochiensis* (Haydock and Evans, 1998a). With a 'high' initial population density of 100 eggs g^{-1} soil, it would take *G. rostochiensis* populations approximately 6 years to decline to non-damaging levels, and *G. pallida* populations about 10 years. The slower decline of *G. pallida* could be related to its ability to store and metabolize food reserves more slowly (Haydock and Evans, 1998b).

Such rates of decline are economically unacceptable to growers and so the most common method of control is to integrate crop rotations with resistant cultivars and nematicides (Evans, 1993). Whitehead *et al.* (1991) found in a sandy loam infested with *G. rostochiensis,* that oxamyl maintained tuber yields in a four-course rotation at the same level as in a six-eight course rotation. Yields of both the susceptible cultivar Désirée and resistant cultivar Maris Piper were also greatly increased with the use of oxamyl.

Nematicides

Nematicides are used on over 52,500 ha in the UK, making up about 0.7 per cent of the arable land (Whitehead, 1986). The decision to apply a nematicide is often based on an estimate of the pre-planting population density (Trudgill *et al.*, 1996). Currently available nematicides belong to two main groups: fumigants and non-fumigants or granular nematicides.

Fumigant nematicides

Fumigants have the advantage of killing nematodes deep in the soil. They work as soil sterilants filling the voids of the soil structure with a gas or vapour which can kill the PCN eggs while they are still contained in the cysts (Williams, 1995). The result is an immediate reduction in population by as much as 90 %. Generally fumigants are applied to field populations exceeding

10 eggs g^{-1} soil. They can be applied at any time in a rotation when no crop is present and soil conditions are suitable. The most common fumigant in the UK is 1,3 dichloropropene (Telone II ®) (Haydock and Evans, 1998b).

Granular nematicides

Granular nematicides are applied and incorporated into the soil immediately before the crop is planted. They protect the most important parts of the young root system during development, so that when PCN finally invade the plant is large enough to tolerate attack (Smelt *et al.*, 1979). Granular nematicides are often preferred due to their specificity, lack of phytotoxicity, easy application in a granular form and application at relatively low rate (Smelt *et al.* 1995). In very heavily infested land some growers use a fumigant nematicide in the autumn to reduce the population followed by a granular nematicide in the spring to protect the growing crop (Haydock and Evans, 1998b). Granular nematicides include *organophosphates* such as ethoprophos, fenamiphos, fensulphothion and thionazin, and *carbamates* such as carbofuran, aldicarb and oxamyl, (Whitehead, 1986).

Cultural control

Biological studies have indicated that nematode populations could be reduced through phenological control, where early planting combined with early lifting could prevent nematodes completing their life cycles and laying eggs (Philis, 1986). Webley and Jones (1981) showed that harvesting potatoes early (83 days after planting) caused a decline in population densities of both species. Harvesting after 91 days permitted some multiplication of *G. rostochiensis* but full multiplication of *G. pallida.* Philis (1986) found a high correlation between the

accumulated day temperatures (ADD) from planting to harvest and nematode multiplication rates on the cultivars Arran Banner and Spunta. It was calculated that nematode populations at harvest could be reduced when the soil ADD, above 10 °C from planting to crop lifting, did not exceed 350 °C. The lifting time is crucial for successful. Reductions in PCN population densities of up to 80 % are possible, but control of *G. pallida* is more difficult as they require fewer day degrees to hatch and develop (Mugniery, 1978). The potential for phenological control is also doubtful as it is unlikely that the higher crop prices and water saving resulting from earlier lifting could balance the loss to yield (Philis, 1986).

Integrated control

For many growers the type of control used in the field is dictated by the multiple retailers, namely supermarkets, who market in excess of 65 % of the fresh potatoes in the UK. Retailers have developed their own integrated crop management (ICM) protocols that refer specifically to PCN management. Some protocols dictate rotation lengths of 1 in 5 years, some specify the use of cultivars resistant to *G. rostochiensis*, however only four cultivars partially resistant to *G. pallida* are mentioned; Nadine, Sante, Rocket and Valor. Other cultivars are often unacceptable because of lower physical or cosmetic qualities such as poor skin finish. The protocols only allow the use of nematicides as part of an integrated control strategy, and are not usually used below population densities of 10 eggs g^{-1} soil because they will not improve the gross margin of the potato crop in which they are used (Evans and Haydock, 1998a).

1.5 Granular Nematicides

Mode of action of granular nematicides

Granular nematicides inhibit the enzyme acetylcholinesterase (ACHE) which is involved in neuro-transmissions within the nervous system. The result is an impairment of neuromuscular and sensory activity, causing in an interference with the locomotion and feeding of the nematode (Evans and Wright, 1982). The sensitivity of AChE in the nematode *Aphelenchus avenae* was three times less sensitive to aldicarb than that in the housefly *Musca domesticus.* This was because the nematode was not as mobile as the insect resulting in reduced contact with the chemical (Pree, 1987).

Hague and Pain (1973) found that treatments at or above 11 kg a.i.ha^{-1} (5 ppm) aldicarb completely prevented multiplication of *G. rostochiensis* and increased yields. This however was above the commercially recommended application rate for aldicarb (Temik®) and oxamyl (Vydate®) of 3.3 and 5.6 kg a.i.ha^{-1}, respectively (Smith and Bromilow, 1977). As a result, instead of implementing a direct lethal action, granular nematicides are described as *nematostatic* as they act by delaying invasion until the plant can tolerate attack (Evans and Wright, 1982).

Once the chemicals are incorporated in to the soil they become dissolved in the soil moisture and are adsorbed through the cuticle of the migrating juvenile. They act by inhibiting the detection of root exudate, causing the juveniles to become disorientated and unable to locate the host. In control plates orientation of the juveniles to the roots was apparent after 4 hours. However when exposed to 2.0 μg ml $^{-1}$ oxamyl, the motility of the juveniles was directly

affected, and at 0.5 μg ml $^{-1}$ oxamyl the nematodes moved randomly with numbers reaching the roots being significantly reduced (Evans and Wright, 1982). Hague and Pain (1973) extracted large numbers of juveniles from soils 4 weeks after treatment with 5 μg g^{-1} aldicarb. The staining of roots after 6 weeks showed fewer nematodes compared with in the control plants. Many of the juveniles had lower food reserves in their intestines suggesting that they were still sufficiently active.

Studies have found the juveniles to be more sensitive to low chemical concentrations than the cysts. At 2.0 μg ml $^{-1}$ oxamyl, juvenile motility was directly affected within 4 hours, however exposure of 1.0μg ml $^{-1}$ oxamyl for 7 days only caused a 50 % suppression of cyst hatching, with an almost complete inhibition at 4.0 μg ml $^{-1}$ oxamyl. *Heterodera rostochiensis* hatching was suppressed at aldicarb concentrations of 2.5 μg ml $^{-1}$, but not at 1.0 μg ml^{-1}. However when exposed initially to root diffusate to stimulate hatching, then transferred to a solution of diffusate and aldicarb at 1.0 μg ml $^{-1}$, a rapid cessation of hatching occurred within 1 week (Osbourne, 1973). This would have been due to the exposure of the sensitive eggs and juveniles within the hatched cysts. Evans and Wright, (1982) suggested that the greater sensitivity of the juveniles could be related to the greater surface area and thus greater surface uptake.

The inhibition of the AChE is a slowly reversible reaction and as the chemical declines the nematodes recover (Pree, 1987). Hague and Pain (1973) found that exposure of 5 μg ml^{-1} aldicarb delayed emergence, however at 6 weeks when the chemical had declined to non-inhibitory levels, nearly 90% of the eggs had hatched. Similarly Evans and Wright (1982) exposed *G. rostochiensis* cysts to solutions containing root diffusate and oxamyl at

concentrations of 4.0 and 16.0 μg ml^{-1} for 7 days. Hatching was almost completely inhibited in the presence of oxamyl, however when transferred to diffusate alone, hatching immediately resumed.

Problems of control

Laboratory studies provide an insight into the mode of action of nematicides however conditions used in the laboratory are carefully controlled and often do not reflect conditions found in the field. Both Evans and Wright (1982) and Osbourne (1973) soaked the cysts in water at constant temperatures of 20 to 24 °C, before exposure to diffusate. The diffusate and nematicides were the only media used, and the experiments were carried out on watchglasses or screw cap vials with constant temperatures, and with Osbourne (1973) the cyst were stored in the dark. Experiments by Hague and Pain (1973) were more realistic as chemicals were applied to pots of soil containing potato tubers and PCN. However pot experiments do not account for the natural variations in leaching and distribution of the nematicide which affect its efficiency in controlling nematodes in the field. As a result, field studies need to be undertaken to determine the actual chemical concentrations controlling PCN in the field.

Nematicide incorporation

Granular nematicides are usually applied as 10 % a.i. (w / w) granules. They do not actively permeate the soil and so thorough mixing is required during incorporation to ensure that an even distribution is achieved (Smelt and Leistra, 1992). Growers often cultivate the granules to a depth of 15 to 20 cm so that when the soil is ridged up the granules will be evenly distributed throughout and between the ridges (Figure 1.06). An uneven distribution could

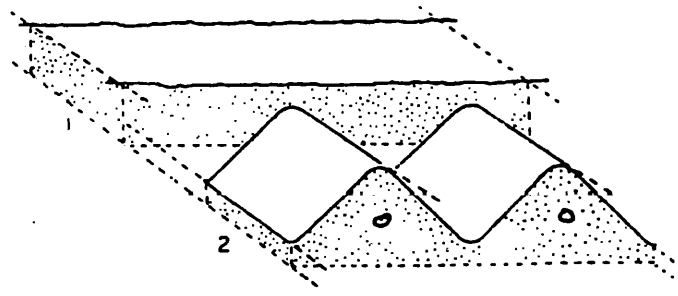


Fig. 1.06 *1. Granules incorporated in the top 15 cm of the soil. 2. Position of granules in the soil after ridging up for potatoes (Whitehead, 1986).*

provide a reservoir of unaffected cysts, which would hatch when developing potato roots reached untreated areas (Whitehead, 1987). Whitehead *et al.* (1975) found incorporating deeper than 20 cm unnecessary as in a moderately infested peat loam, yield was increased as much with aldicarb incorporated to 13 cm depths as it was to 25 or 38 cm. Mixing to greater depths would also be costly, and in soil with a high water table is undesirable due to the risk of ground water contamination (Whitehead, 1987).

Smith and Bromilow (1977), achieved a better distribution of chemical to the working depth using rotavators compared with harrows, the latter leaving 60-70 % of the granules in the top 5 cm. Granular nematicides can also be applied with a stone separator, removing the need for separate application and incorporation. However Woods *et al.* (1999) found satisfactory incorporation was achieved when the stone separator was at the share or first web interface but shallow incorporation (approximately 5 cm) when it was halfway up the web. The combination of initial granule incorporation by bed tilling followed by a second incorporation by stone separation produced a deep and potentially poor distribution (approximately 35 cm).

Distribution of granular nematicides

Once incorporated into the soil granular nematicides need to be redistributed by leaching from rainfall or irrigation to protect the most important parts of the root systems (Smelt, 1992). The speed of redistribution is limited by the properties of the chemical, the type of granule in which it is incorporated, soil type and the weather, especially rainfall (Bromilow, 1973).

Adsorption

The adsorption of a chemical is often expressed as the coefficient K_{om}, which determines the partitioning of the chemical between soil water and soil organic matter in a dynamic equilibrium (Figure 1.07). The amount of adsorption is determined by the structure of the chemical and the organic

$$K_{om} = \frac{\textit{Chemical concentration in soil organic matter (mg/kg)}}{\textit{Chemical concentration in soil water (mg/dm}^3\textit{)}}$$

Fig. 1.07 *The K_{om} coefficient (Smelt and Leistra, 1992)*

matter content of the soil, with the concentration of nematicide in soil solution being highly reduced with increasing organic matter (Bromilow, 1973). Table 1.01 displays the K_{om} values for a number of pesticides (Smelt and Leistra, 1992). The lower the K_{om} value the less the adsorption and so the greater the potential mobility of the chemical.

On a sandy loam, aldicarb was slightly more strongly adsorbed than oxamyl, whereas aldicarb sulphoxide and sulphone were slightly more weakly adsorbed than oxamyl. (Bromilow *et al.*, 1980). This weak adsorption makes aldicarb and oxamyl suitable for use in a wide range of

soils including humic and peaty. Other nematicides such as carbofuran, ethoprophos and fenamiphos are more highly absorbed and will not be leached as well (Smelt and Leistra, 1992).

Compound	K_{om} (dm^3 / kg)	References
Aldicarb	5 – 13	Bromilow et al., (1980)
A. sulphoxide	0 – 4	
A.sulphone	2 – 6	
Carbofuran	17 – 37	Felsot and Wilson (1980)
Ethoprophos	43 – 68	Leistra and Smelt (1981)
Fenamiphos	100 – 141	Bromilow (1973)
Oxamyl	2 – 7	Bromilow (1973)

Table 1.01 *Coefficients (K_{om}) for adsorption of some carbamate and organophosphate nematicides and their bio-active products on soil organic matter (Smelt and Leistra, 1992).*

The type of crops grown between rotations will effect the organic matter content of the soil. One year of grass growth was shown to increase the organic matter by 0.2-0.3 % in the top 200 mm of a soil compared with less then 0.1 % for potatoes (Davies *et al.*, 1993).

Water movement

In spring when most nematicides are applied, the net downward water flow is highly reduced resulting in limited leaching below the depth of incorporation. Only in short periods with substantial rainfall will there be a clear downward transport and in drying periods there may even be upward movement (Smelt and Leistra, 1992). When moisture is readily available to the plant in the top soil, damage to the deep roots is much less important. However if there is a low water table and the crop is not irrigated in periods of drought, damage to the deeper roots may be important (Whitehead, 1987). The available water capacity (AWC) of a soil gives a useful

indication of whether or not a water shortage is likely to reduce yields. The AWC is the quantity of water held in the soil between the limits of field capacity (point at which drainage begins) and permanent wilting point (Davies *et al.*, 1993).

Whitehead *et al.* (1991) found that 5.6 kg ha $^{-1}$ oxamyl and aldicarb applied to a moderately infested sandy loam (65 eggs g^{-1}) controlled *G. pallida* well, however in some peaty loams poorer control of *G. pallida* was found. This was believed to be due to the lower water holding capacity of the sandy loam which caused the hatched juveniles and males to be exposed to more concentrated levels of chemical for longer. Pesticides in soils containing high levels of organic matter are leached less and so deeper incorporations of the nematicides may be necessary for good control of the nematode (Whitehead *et al.*, 1981).

Soil type and structure

A chemical will move less in a finely structured than in a coarsely structured soil because equilibration of the chemical between water and the interior of soil aggregates becomes slower with increasing aggregate size (Bromilow, 1973). This effect is shown in Figure 1.08, which was reconstructed from the studies of Leistra and Smelt (1981). In a silty and sandy loam with a low organic matter content (1.6 and 1.8 %), ethoprophos was leached distinctly deeper than in a loamy sand with a much higher organic matter (6.1 %), and consequently higher adsorption. Water movement is primarily vertical in soils with a light sandy texture, for heavier soils there is a greater degree of horizontal water movement and shallower penetration (Gerstl, 1984).

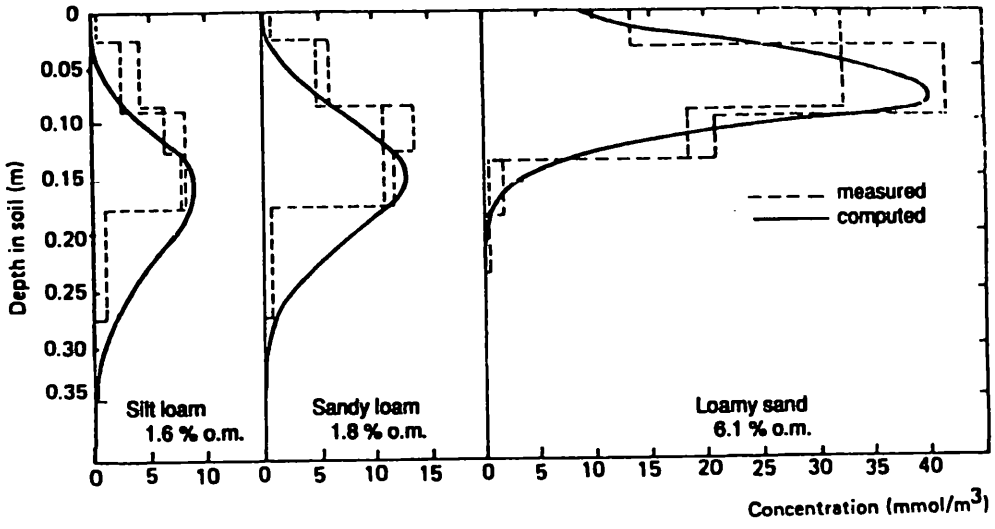


Fig. 1. 08 *Simulated and measured concentrations of ethoprophos in three soils under field conditions during winter. Granules incorporated 0.1 m deep. Results after 139 days with 210 mm rainfall, o.m. = organic matter (Smelt and Leistra, 1992).*

Degradation of nematicides

Most approved nematicides show a moderate persistence in the soil of several weeks. However from an environmental perspective long-term persistence is not desirable as it may lead to toxic residues in the potatoes, leaching to groundwater supplies or long lasting side effects (Smelt and Leistra, 1992). Since the phasing out of persistent chemicals such as aldrin and chlordane in the late 1970s (Racke, 1990), soil microorganisms have become important in the degradation of more biodegradable chemicals, metabolising the toxins into less harmful by-products (Felsot, 1989).

For some chemicals the breakdown is important for the implementation of control. Normeyder and Dickson (1990) found that carbofuran was an approximately 20,000-fold more potent AChE inhibitor than fenamiphos in the root knot nematode *Meloidogyne incognita*, however biological data demonstrated a 10-fold higher efficacy of fenamiphos over carbofuran. This was

because carbofuran is rapidly metabolised into less active degradation products, whereas fenamiphos is relatively stable within the nematode and is only degraded into even more potent fenamiphos sulphoxide.

The breakdown of aldicarb is similar to that of fenamiphos. The parent compound is rapidly oxidised to its sulphoxide, which in turn is oxidised more slowly to aldoxycarb (aldicarb sulphone), before being hydrolysed to non-toxic oximes (Figure 1.09). The oxidation processes are accompanied by some hydrolysis (Bromilow, 1980).

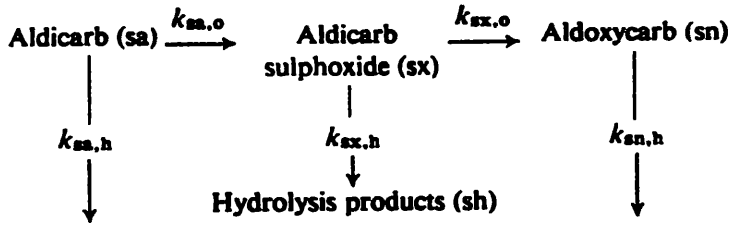
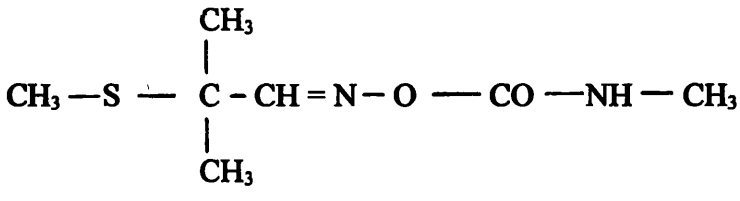


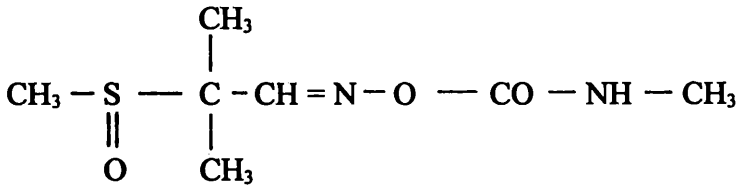
Figure 1.09 *The oxidation and hydrolysis pathways of aldicarb degradation*

Nordmeyer and Dickson (1990) reported that the aldicarb sulfoxide was 10 to 22 times more inhibitory than the parent aldicarb, with the sulphone being even less inhibitory than the aldicarb. Figure 1.10 shows the chemical structures of aldicarb, its metabolites and oxamyl.

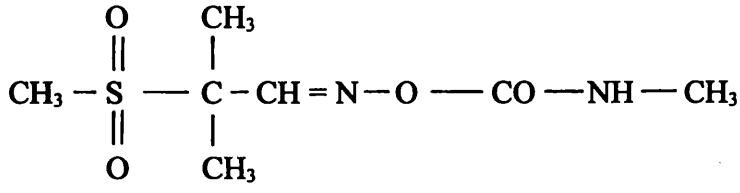
Aldicarb 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyloxime)



Aldicarb sulphoxide, 2-methyl-2-(methylsulphinyl)propionaldehyde 0-(methylcarbamoyl)oxime



Aldicarb sulphone, 2-methyl-2-(methylsulphonyl)propionaldehyde *0*-(methylcarbamoyl)oxime



Oxamyl, *N,N*-dimethylcarbamoyloxyimino-2-(methylthio)acetamide

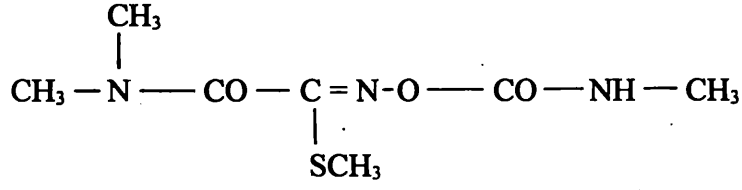


Figure 1.10 The chemical structure of aldicarb its two oxidation products and oxamyl (Bromilow, 1980)

Normeyder and Dickson (1990) also found oxamyl to be very inhibitory to the nematode AChE however the half-life of oxamyl is relatively short as the compound is directly degraded by hydrolysis of the carbamate group to non toxic oximes. The processes involved in the breakdown of oxamyl and aldicarb closely follow first order rate kinetics (Bromilow *et al.*, 1980), however the rate of breakdown can vary dramatically depending on soil type, temperature and moisture content of the soil (Leistra *et al.*, 1980).

Effects of moisture

Smelt *et al.* (1979) found that in a humic loamy sand and clay loam at 15 °C, decreasing the moisture content of the soil to about wilting point slowed the rate of conversion of oxamyl. Similarly Gerstl (1984) found that as water in the soil increased from 3.5 – 16.6 % moisture content, the transformation of oxamyl also increased up to approximately 60 % field capacity when conversion rates leveled off. The effect of moisture content on the decomposition of oxamyl could be a reflection of the effects on microbial activity. As the soil dries out, it supports a smaller microbial population, and hence there is a decrease in the decomposition of organic molecules (Gerstl, 1984).

Temperature

Gerstl (1984) found temperature to follow the Arrhenius relationship with degradation proceeding more rapidly at higher temperatures of 35 °C compared with 15 °C. This range was chosen to cover the range of soil temperatures that may occur in Israeli soils during the growing season. Smelt *et al.* (1978) found that in a clay soil and greenhouse soil incubated at 6, 15 and 25 °C higher temperatures greatly affected the rate of conversion of aldicarb sulphoxide with conversion rates of 0.009, 0.033 and 0.05 day^{-1} respectively. Dukes *et al.*(1996) found that in a sandy loam, degradation rates increased with increasing moisture and temperature. At low moisture contents, equal to 40 % AWC, high temperatures of 20 °C had less of an impact on transformation rates than at the higher moisture content of 75 % AWC. Similarly, at low temperatures of 8 °C the higher moisture contents had a lower impact.

pH

Smelt *et al.*, (1979) found in incubation studies at 15 °C that the half life of oxamyl ranged from 13 to 14 days in a clay loam and loamy sand, and 34 to 39 days in a peaty sand and humic loam sand, respectively. They attributed the difference in the rates of degradation to variations in the pH of the soils. With the former two soils the pH was 7.1 and 7.4, compared with 5.4 and 5.2 at the latter sites. Harvey and Han (1979) reported that the stability of oxamyl in aqueous solution was considerably less at a higher pH than at pH 4.7, and so the faster conversions of 13-14 days could have been related to the higher pH of the soils. Other studies have confirmed this. In an acidic peaty sand (pH 4.5) the mean half life of ethoprophos was 87 days, whereas under alkaline conditions (pH 7.2) the half life was reduced to 14-28 days (Smelt *et al.*1977). Aldicarb, oxamyl and ethoprophos degraded quicker in a sandy soil of pH 7.3 than in a sandy soil of pH 5.6 (Smelt *et al.*, 1996). Degradation occurs faster in alkaline soils due to increased levels of hydrolysis. Generally nematicides and their bio-active products have the highest degradation rates in a loamy soils with a high pH, and the lowest rates in organic soils with a low pH (Smelt and Leistra, 1992).

1.6 Accelerated Degradation

Accelerated (enhanced) degradation is described as the accelerated dissipation of a chemical after repeated applications of the compound to the same soil (Felsot, 1989). The phenomenon is believed to be the result of microbial degraders in the soil which after years of exposure to the same or similar compounds, have physiological adapted themselves to be able to rapidly utilise the chemical as a nutrient or energy source (Racke, 1990).

The first incidence of accelerated degradation was reported 40 years ago for 2,4-dichlorophenoxyacetic acid (Audus, 1949). However the implications of the development of enhanced degradation for crop protection practises were not realised until the early 1970s when important insecticides began to fail in their control of corn rootworm in the USA (Felsot, 1989). Failures in the field performance of the herbicide EPTC and of the insecticide carbofuran were correlated with the rapid loss of their residues in previously treated soils. Corn continues to be the largest single crop in the USA and so threats of accelerated degradation could be devastating. In the UK and Europe there have been no indications of accelerated degradation becoming a major threat to cereals and the agrochemical market. Furthermore, with the phenomenon restricted almost by definition to those pesticides which are applied directly to the soil, only a small proportion of compounds are likely to be exposed to its consequences. However, this limitation emphasises the severity of the threat to growers of crops liable to damage by soil inhabiting pests, particularly as the armoury of pesticides available to control these pests is not large, and is unlikely to increase significantly in the near future (Suett, 1990).

Theoretical aspects of accelerated degradation

Knowledge of the mechanisms by which soil microflora become adapted for the biodegradation of insecticides is important for understanding the practical aspects of the relationship between enhanced biodegradation and the control of insect pests (Felsot, 1989). The processes of degradation following herbicide applications are shown in Figure 1.11. Initially a small but fairly rapid decrease in herbicide concentration occurs due to soil sorption processes. This is followed by a lag phase where no appreciable change in concentration occurs. At the end of this phase there is a period of rapid substrate disappearance (Kearney and Kellogg, 1985).

The lag phase appears to be the time required for specific pesticide-degrading enzymes and coenzyme to be synthesised (Racke, 1990). Subsequent additions of the same or chemically related herbicide resulted in a more rapid disappearance of the chemical with no recognisable lag phase. This was because the degrading population had adapted itself to attain a critical size to degrade the pesticide at a reasonably rapid rate (Kearney and Kellogg, 1985).

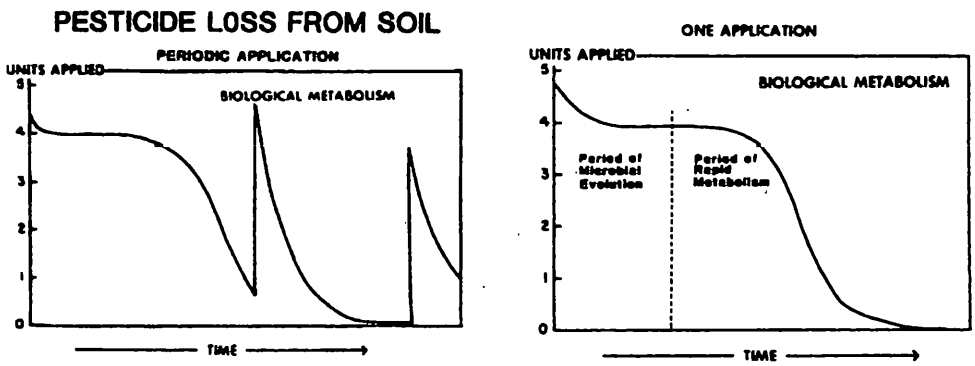


Fig 1.11 A kinetics of pesticide biodegradation B, Periods of evolution and rapid metabolism of a biodegradable pesticide (Kearney and Kellogg, 1985)

Moorman (1988) found in soils with low and infrequent applications of the herbicide EPTC accelerated degradation occurred however no appreciable increase in the number of degrading populations was observed. It was suggested that the less frequent additions of the chemical could have been insufficient to support population growth indeed substrate energy supply must exceed that required for maintenance of existing biomass for populations to grow. As a result the accelerated rates of breakdown would indicate that the adaptation process was physiological leading to increased activity of herbicide-degrading enzymes, rather than due to increased populations of degraders.

Studies characterising the microbial degraders have found them to possess distinct plasmids containing hydrolysing enzymes, which are believed to be responsible for the accelerated degradation (Kearney and Kellogg, 1985). Molecular studies suggest that the development and spread of pesticide degrading enzymes originated from the catabolisers of natural substrates. Through competition for resources these enzymes could have diverged and evolved to enable the catabolism of synthetic compounds. Plasmids isolated from *Flavobacterium* and *Pseudomonas* species were found to be evolutionarily distinct, however the hydrolysing enzymes were homologous suggesting they came from a common source (Head *et al.*, 1990). Plasmids are independently replicating minichromosomes. In addition to specific internal DNA recombinations, external DNA additions can occur via plasmids between various soil bacteria. It is feasible that accelerated degradation is the result of an acquired ability to degrade toxins faster, through the spread of hydrolysing enzymes by specific microbial strains (Kearney and Kellogg, 1985).

Practical aspects of accelerated degradation

Over the last decade an increasing number of studies have been carried out, using many different compounds, to compare the degradation of a pesticide in soils previously treated with the chemical with soils which had never been treated. The results of one study are shown in Figures 1.12 and 1.13 where Smelt *et al.* (1987) measured the transformation of aldicarb and oxamyl in incubation studies at 15°C and moisture pressure of -10^4 Pa. Results showed that the nematicides decreased more rapidly in the previously treated soils.

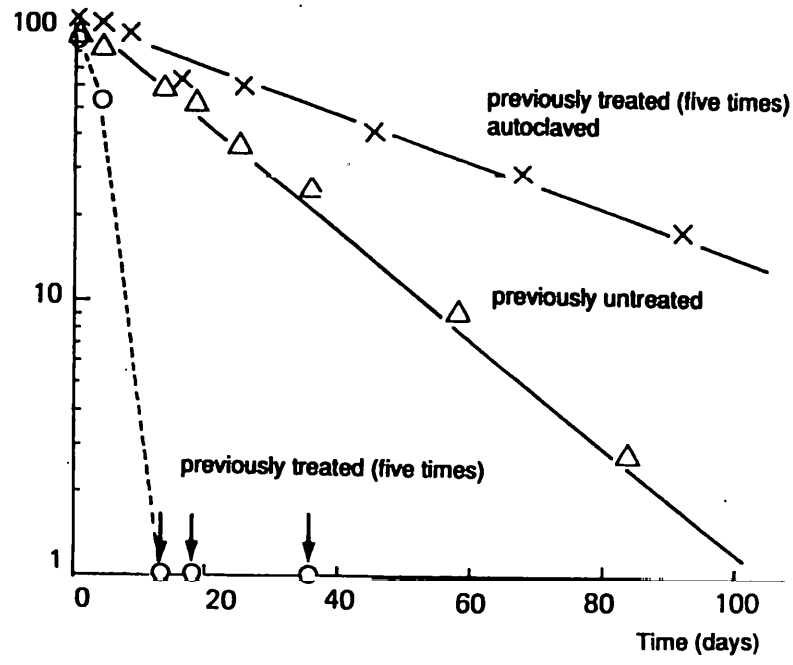


Fig 1.12*Transformation of oxamyl in soil samples (sandy loam) from annually treated and non-treated plots. Incubation studies at 15 °C (Smelt et. al., 1987).*

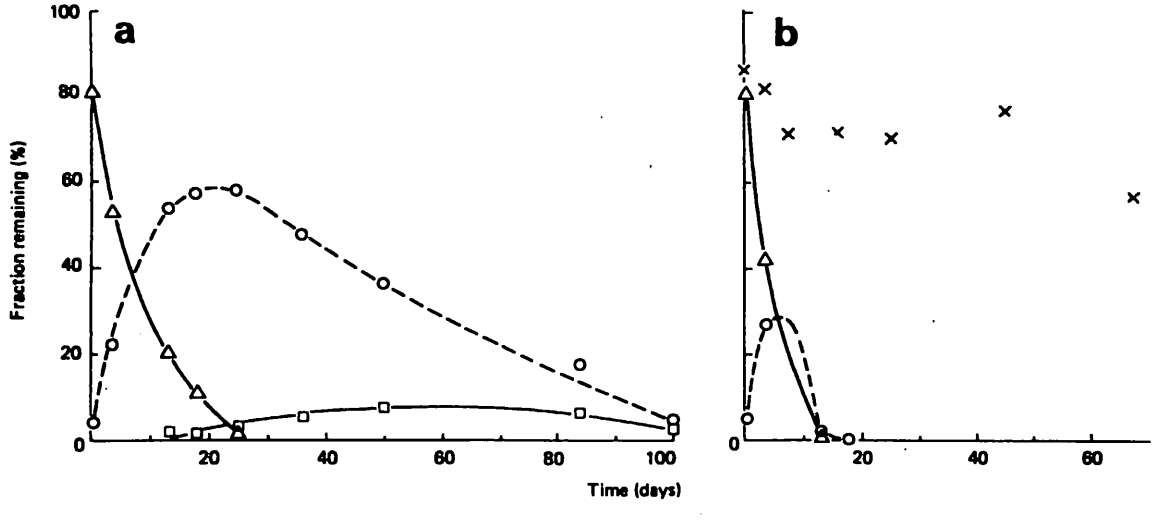


Fig. 1.13 *Disappearance of aldicarb and formation and disappearance of its oxidation products in soils from (a) previously treated and (b) four times treated plots at 15 °C △, aldicarb; 0, aldicarb sulphoxide; □, aldicarb sulphone; ×, aldicarb in autoclaved soil of treated plots.*

Sterilisation of the treated soils by autoclaving at 120 °C drastically reduced the rates of disappearance. Smelt *et al.* (1987) concluded from this that microbial processes were probably

involved in the rapid transformation of the treated soils. Stirling *et al.* (1992) found that in gamma-irradiated or autoclaved soils significant activity of fenamiphos was retained for up to 6 weeks, whereas in untreated soils the activity was lost within 2 weeks. They also concluded that biological degradation was the most likely cause of the loss in efficacy, particularly as there was an increase in the rate of degradation of fenamiphos as proportions of untreated soil were added to the autoclaved soil.

Suett and Jukes (1988) obtained treated and untreated soils from sites in England where aldicarb had been applied in at least one previous year (histories of the soils are shown in Table1.02). The soils were treated with aldicarb at 25 mg a.i. kg^{-1} dry soil, and incubated at

Soil no.	Insecticide	Dose rate	Application frequency
1 A	Aldicarb	2.8 – 4.0 kg a.i. ha^{-1}	Once per 1-2 years for 8 years
U	Untreated		
2 A	Aldicarb	0.75 g a.i. / m^2	Once per year for 3 years
U	Untreated		
3 A	Aldicarb	0.8 g a.i. / stock	Once in 1985
U	Untreated		
4 A	Aldicarb	6 kg a.i. ha^{-1}	Once per year for 13 years
U	Untreated		
5 A	Aldicarb	50 mg a.i. m^{-1} row	Once in 1986
B	Aldicarb	105 mg a.i. m^{-1} row	Once in 1986
C	Aldicarb	222 mg a.i. m^{-1} row	Once in 1986
D	Thiofanox	165 mg a.i. m^{-1} row	Once in 1986
E	Carbofuran	171 mg a.i. m^{-1} row	Once in 1986

Table 1.02 *Treatment history of soils studied in incubation experiments*

15°C and 33 kPa moisture. Despite widely different histories of nematicide usage, in soils 1 to 4 total aldicarb residues were always lost more quickly from previously treated soils than from the corresponding untreated sample. With the treated soils the time for initial 50 % loss ranged from 5 to 17 days whereas in the untreated soils the range was 25-50 days. Studies with the herbicide MCPA also found similar differences between treated and untreated soils (Figure 1.14)

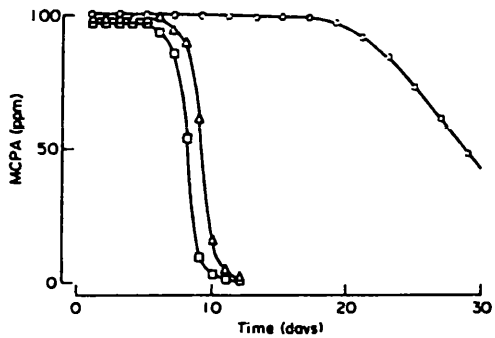


Fig. 1.14 *Degradation of MCPA from a field experiment with annual applications of MCPA for 0 (o), 1 (Δ), and 18 (□) years. Samples were taken 9 months after the preceding application (in Kearney and Kellogg, 1985).*

Hendry and Richardson (1988) showed an increase in the number of carbofuran-hydrolyzers following an initial treatment with carbofuran to previously treated soils. Population growth was measured as the most probable number (MPN) which measured the amount of radioactivity (made up as 0.25 m NaOH and scintillation cocktail) evolved with CO_2 release from the microbes. The MPN was measured as 310,000 in treated soils, compared to only 1600 in untreated soils. Charnay and Fournier (1994) found that most soils with a history of carbofuran usage had a pesticide degrading population exceeding the detection threshold, with some soils containing more that 10 000 carbofuran-degrading bacteria per gram of soil. In contrast, only 44 % of the soils never treated with methylcarbamates contained degrading micro-organisms exceeding this level.

Suett and Jukes (1988) observed a dose-related response in soils 5A, 5B and 5C (Table 1.12) with the aldicarb declining most rapidly from the soil treated with the largest dose. Eight weeks after re-treatment 5C (222 mg a.i. m^{-1} row) contained less than 20% of the applied dose compared with 70% and 78% in 5A (50 mg) and 5B (105 mg). Suett (1986) also found that carbofuran degradation was proportionally slower at 2 mg kg^{-1} than at 10 and 50 mg kg^{-1}.

The development of accelerated degradation would also appear to be influenced by frequency of treatment. Hendry and Richardson (1988) found that soils did not show rapid degradation of carbofuran three weeks following a pretreatment of 9 $\mu g.g^{-1}$. However when treated a second time four weeks after the pretreatment, 63 % was hydrolysed within 3 days and after a third treatment 278 h after the second, 92% of the carbofuran was hydrolysed within 19h. Charnay and Fournier (1994) also found that an initial treatment of carbofuran produced an average half-life of 17 days, however after a second treatment the average half life was shortened to 8 days.

Studies have also shown that the degradative capacity of soils can persist for some time. Results from studies using fenamiphos showed microbial degraders still present in the soil three years after the last nematicide application (Stirling *et al.*, 1992). Morel-Chevillet *et al.* (1996) also found a degrading capacity to be persisting in a soil five years after carbofuran usage and Parekh *et al.* (1992) found that soils collected three months after carbofuran treatment were degraded at similar rates to those collected four years later. The persistence of microbial adaptation probably depends upon the persistence of the pesticide and the ability of the enriched microbial population to survive and remain active in the absence of the pesticide or in the presence of very low levels. Microbial degrading populations may be able to survive and

remain active for some time after the pesticide is completely degraded by utilising naturally occurring compounds as alternate substrates for the pesticide degrading enzymes (Hendry and Richardson, 1988).

Accelerated degradation does not occur in all soils. Smelt *et al.* (1996) found a highly accelerated rate of degradation after three and five applications of oxamyl and aldicarb in a soil of pH 7, however in another soil of pH 5.6 degradation increased only slightly after 10 applications. Suett *et al.* (1996) suggested that factors such as pH and adsorption could limit the activity of microflora indirectly by favouring abiotic transformations or by reducing the bioavailability of pesticides, while soil organic matter could modify the type and number of pesticide degrading microbes. The resistance of some soils to accelerated degradation could also be a result of scarcity or poor survival of adapted strains (Charnay and Fournier, 1994). For the carbamates many different degrading strains have been described, with isolates from one soil often differing from those from another soil (Suett *et al.*, 1996).

In the studies of Suett (1986), despite the ability of all the treated soils to degrade carbofuran rapidly in the laboratory, all except one of the growers who co-operated were satisfied with the effectiveness of a granular fomulation of carbofuran in the field. Similar responses were found with aldicarb (Suett and Jukes, 1988). Such conflicting data stresses the importance of corroborating laboratory studies with field observations, as in the laboratory soils are maintained at a constant moisture and temperature, conditions never obtained in the field (Suett and Jukes, 1988). However it should also be remembered that although the growers did not report a field problem at the time, the results of the studies proved the *potential* for accelerated degradation. In the Netherlands frequent use of aldicarb, oxamyl and ethoprophos has led to

greatly-reduced efficacy, and on occasions complete failure of control of the potato cyst nematode has occurred (Smelt *et al.*, 1987).

Cross adaptation

A final phenomenon has been described as cross-adaptation, where previous use of a certain pesticide induces the accelerated transformation of *another* pesticide, usually with a related chemical structure (Smelt and Leistra, 1992). Smelt *et al.* (1987) found that the disappearance of oxamyl was distinctly faster in a loamy soil pretreated 5 times with aldicarb, compared with a soil treated five times with the organophosphate ethoprophos. Transformation of aldicarb was also distinctly more rapid in soils previously treated with oxamyl than with ethoprophos (Figure 1.15).

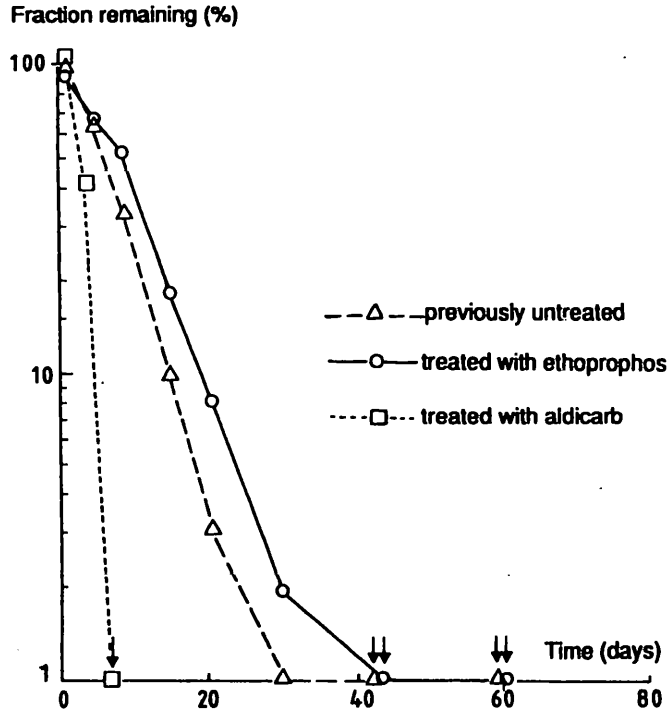


Figure 1.15 *Effect of previous treatments (five times) with ethoprophos and aldicarb on the transformation rate of oxamyl (Smelt et al., 1987).*

Corresponding results were reported by Harris *et al.* (1984) who found that in incubation studies with carbofuran-adapted soil, the rate of disappearance of the organophosphate, phorate was not affected by previous treatments with carbofuran, however a variety of carbamates, including aldicarb and oxamyl, had increased rates. Morel-Chevillet *et al.* (1996) found that prior treatments with 15 *N*-methylcarbamates led to an enhanced rate of carbofuran degradation and an increase in the number of carbofuran–degrading microorganisms.

These studies emphasise the importance of avoiding even trace contamination of compounds with other soils, particularly as Harris *et al.* (1984) found the ability to degrade carbofuran rapidly could be induced in a previously untreated soil by transferring as little as 0.25% of an active soil. Studies also promote the potential for alternating organophosphates with oximecarbamates as a way of preventing accelerated degradation, and ensuring the persisting control of PCN in UK soils (Smelt *et al.*,1984).

1.7 OBJECTIVES OF THE THESIS

Research for this thesis will be examining the degradation of soil-applied nematicides and the effects on the control of potato cyst nematodes.

The aims of the investigation are to test the hypothesis that:

- Soil moisture content, temperature, and microbial populations affect the rate of degradation of granular nematicides.

- The rate of degradation of granular nematicides directly relates to the control of *G. rostochiensis* and *G. pallida* populations in the soil.

- Nematicide biodegradation in the laboratory is a reliable method for studying the degradation rates of field soils.

- Previous treatment of soils with granular nematicides results in the decreased persistence of subsequent nematicide applications.

Chapter 2

The rate of degradation of oxamyl (Vydate) in field soils

2.1 INTRODUCTION

In the last decade an increasing number of studies have reported a decreased efficiency of soil applied nematicides after repeated applications to the same soil. Any enhanced degradation of low persistence carbamate nematicides would particularly affect the control of *G. pallida* populations where the period of hatching can last up to four weeks longer than for *G. rostochiensis*. This could result in *G. pallida* still emerging when nematicides have degraded to concentrations below the minimum required to paralyse juveniles in the soil.

The movement and degradation of agricultural chemicals in the environment is a complex process influenced by application variables, field variables such as soil pH; and edaphic factors such as soil temperature and moisture content (Suett, 1990). As a result the majority of enhanced degradation studies have been investigated under laboratory conditions.

In laboratory studies the half-life of the chemical is used to compare rates of degradation. However, under field conditions the half-life can not be predicted as constant fluctuations in moisture and temperature result in daily variations in the breakdown of the chemical (Jones, 1992).

Objectives of the trial

- To estimate degradation rates of oxamyl in Shropshire soils.

- To quantify factors such as previous treatment history, microbial population, soil water content and temperature which may affect the persistence of the chemical.

- To establish the validity of using mathematical models to predict the disappearance time (Dt_{50}) of oxamyl in the field and help understand the environmental fate of the chemical at each site.

2.2 MATERIALS AND METHODS

2.2.1 Site selection

Ten farms in the Shropshire area were selected, within a fifteen miles radius of Harper Adams University College, where oxamyl was to be applied as Vydate to a potato crop. Areas within the field with the highest estimated population density of PCN were used for sampling. Data was obtained from the growers who had employed commercial soil samplers in previous years. Farming practices such as methods of oxamyl incorporation, planting data and previous cropping history are shown in Tables 2.1 to 2.3. This information was obtained from questionnaires sent to the growers (Appendix 2.1).

Four stations, (5 x 5 metres) were marked out using canes, on the areas of the field with the highest estimated population densities. Soil and plant samples were taken during the week of planting, and then at weekly intervals for 13 weeks. Thirteen markers were randomly placed into the ridges at each station to ensure that samples were taken from undisturbed soil each week.

Soil sampling

Each week a single plant was lifted from each station. Before removal, four soil cores were taken from undisturbed ridge soil around the plant. The cores were taken using a cheese-type auger to a depth of 300mm x 25mm and bulked in a labelled polypropylene bag. The depth of the core enabled detection of the chemical to continue when displacement down the profile by leaching had occurred. The samples were immediately frozen at –14 °C to prevent further degradation of the nematicide occurring. The extracted plants were used for root invasion studies, which are described in Chapter 3.

Site	rate of Vydate applied (kg/ha)	incorporation technique	destoner used?	planting depth (cm)	irrigation dates and application rates (mm)	
					date	rate
Bigwood	40	S-tined cultivator	no	15-20	late June, 8 th, 16 th July	13 mm
Stockton	55	Spiked rotary cultivator	yes	8	21-May	20 mm
Heaths	54	Power harrow	no	12	8th, 15th, 24th May - 4th, 18th, 25th July	2 x 15 mm, 3 x 20 mm
Round	55	Spiked rotary cultivator	yes	17-18	8th, 17th July	20 mm
Common	55	Spiked rotary cultivator	yes	14	4 times (dates unknown)	25 mm
Searchlight	55	Bed tiller	yes	15	25-May 11-July	15 and 22 mm
Front	37.1	Applied on destoner	yes	13 cm	17th July	25 mm
Crab	54	Power harrow	no	12	3rd, 18 th, 24th July	15, 20, 20 mm
Malt	40	S-tined cultivator	no	15-20	19th, 30th May	13 mm
Otter	37.1	Applied on destoner	yes	13 cm	23rd May, 7th June, 15th July	2 x 25 mm, 1 x 20 mm

Table 2.1 *Incorporation data from each site*

Site	rotation length (years)	planting date 1998	potato cultivar grown	resistance to *G.rostochiensis?*
Bigwood	3	02-Apr	Dundrod	yes
Stockton	3	02-Apr	Premiere	yes
Heaths	4	20-Mar	Maris piper	yes
Round	4	22-Apr	Maris piper	yes
Common	4	01-Apr	Maris piper	yes
Searchlight	5	30-Mar	Estima	no
Front	4	02-Apr	Maris piper	yes
Crab	4	08-May	Maris piper	yes
Malt	3	19-Mar	Maris bard	no
Otter	4	17-Mar	Maris piper	yes

Table 2.2 *Planting data from each site*

Site	number of years potatoes grown	Previous cropping			
		1997	1996	1995	1994
Bigwood	33 +	Beet (aphox twice)	winter wheat (aphox once)	potatoes	
Stockton	*	beet	grass, maize	potatoes	
Heaths	20 +	wheat (cyphermethrin)	beet (granular seed dressing)	wheat (-)	
Round	50	wheat (aphox)	beet (aphox)	flowers	
Common	50 +	wheat (cyphermethrin and dimethoate)	beet cyphemethrin and pirimicarb)	wheat (cyphermethrin and dimethoate)	
Searchlight	30 +	grass (-)	wheat (-)	beet (cyphermethrin)	barley (-)
Front	*	beet	barley	wheat	
Crab	25 +	wheat (cyphermethrin)	beet (granular seed dressing)	wheat (-)	
Malt	33 +	Beet (aphox twice)	barley wheat (aphox once)	potatoes	
Otter	*	wheat	barley	carrots	

Table 2.3 *Cropping data from each site*

( ) = chemicals applied to crops
(-) none used

* = unknown information

2.2.2 Soil analyses

Particle size analysis (mechanical analysis)

Methods used closely followed those described by MAFF/ADAS (1986). Soils were initially sieved through a 2mm mesh, and 10 gram sub-samples weighed into a 600 ml beaker. Next 20 % aqueous hydrogen peroxide solution (400 mm^3) was added to oxidise the organic matter from the soil. The beakers were warmed gently on a hot plate for 20 minutes, stirring occasionally, then boiled for a further 5 minutes. Following this dispersing agent (100 mm^3), was added to the soil suspension (Appendix 2.2).

The soil from each beaker was transferred to a 500 ml bottle, made up to 150 ml with distilled water, and shaken for 5 minutes. The solution was then poured through a 0.063mm sieve into a 500 ml measuring cylinder. A jet of distilled water was used to push smaller particles through the sieve. The sieve contents was transferred to an evaporating dish and dried in an oven at 105 °C for 24 hours. The measuring cylinder was made up to 500ml with distilled water, stirred, and then 25ml of the suspension immediately removed from a depth of 200 mm below the liquid surface. This was also placed into an evaporating basin and dried at 105 °C for 24 hours. This comprised the silt and clay fraction.

After 24 hours the contents of the dried sieve were transferred to a bank of sieves comprising of a: lid, 0.6 mm, 0.212 mm, 0.063 mm sieves and a receiving tray. The sieves were shaken for 10 minutes and any material in the receiving tray put into the measuring cylinder. Each sieve fraction was then transferred to a watch glass and weighed. This was the sand fraction. The contents of the measuring cylinder was re-mixed and left to

sediment for 8 hours. After this time a further 25ml was removed to a depth of 90 mm and placed in a weighed evaporating dish at 105 °C for 24 hours, this was the clay fraction. Calculations for the percentage of sand, silt and clay are shown in Appendix 2.2. The soil type was assessed using a texture triangle (Hodgson, 1976).

Organic matter content

Ten grams of soil was weighed into a crucible and placed in an oven at 105 °C for 24 hours. The samples were then cooled in a desiccator over silica gel and weighed to give the moisture content of the soil. The crucible was then placed in a furnace at 500 °C for 18 hours cooled and re-weighed to give the organic matter content. Methods of calculation are shown in Appendix 2.2.

pH

Ten grams of sieved soil were placed in a 50 ml bottle with 25ml of water. The bottles were then shaken at 300 rpm for 15 minutes on an IKA Labortechnik shaker. The soil suspension was then stirred with a glass rod and the electrodes from a pH metre, (model Russel mode RL150) were used to record the pH of the soil.

2.2.3 Recording the temperature and rainfall

Temperature

Constant monitoring of the soil temperature was achieved using a Gemini Tiny Talk data-logger, model OTLM, IP-68. This was buried to a depth of 150 mm in the middle of a bed in the selected site. Logging began on the day of planting with the temperature taken every 1 hour for 13 weeks. The mean soil temperature per day was calculated from the minimum

and maximum temperatures. From this data the accumulated heat in day degrees (ADD) was calculated (Appendix 2.3).

Rainfall

Rainfall data was obtained from Shawbury Meterological Station, Shawbury, Shropshire.

2.2.4 HPLC Analyses

Oxamyl extraction

The extraction technique enabled 16 soil samples to be analysed per day, with 3 replicates per sample. At approximately 5pm the evening before extraction, the frozen soils were placed onto metal trays to defrost. Sub-samples (approx. 60g soil) were removed from the frozen blocks using a hammer and resealed into bags to be used for moisture analyses.

The next morning the soils were sieved through a 2mm mesh, and 3 x 20g sub-samples weighed into numbered 100 ml jars. To each jar 20 ml of analytical grade methanol was added and the jars were shaken on a Labortechnik shaker at 300 rpm for 3 hours. The soil solutions were then filtered through a funnel containing a number 5 Whatman filter paper, into a conical flask. Using a 1 ml syringe, the filtrate was further cleaned by passing through a 0.45μm filter into a labelled 2mm vial. The oxamyl concentration was then measured using high pressure liquid chromatography (HPLC).

Moisture contents of the soils

The soils were removed from the bags and sieved through a 2 mm aperture. Three 10g samples were placed into weighed foil lids and heated in an oven at 105 °C for 24 hours. The soils were then removed, re-weighed and the moisture contents calculated (see Appendix 2.2).

High Performance Liquid Chromatography (HPLC) analysis

A Hewlett Packard Series 1100 HPLC was used for the analyses. The column was a Phenomenox 250 x 46 mm Sphereclone 5μ ODS and was held at 30 °C. The mobile phase was 50:50 HPLC grade water and methanol run at a 1.2 ml min^{-1} flow rate. A 20μl injection was monitored at 220nm wavelength with a 3.5 minutes retention time.

Analytical efficiency

To validate the efficiency of the technique the percentage recovery of oxamyl was established. A 1000 μg ml^{-1} standard was made using 50 mg of 99 % pure oxamyl granules in 50 ml of analytical grade methanol. From this a 50 μg ml^{-1} standard was made using 2.5ml of the 1000 μg ml^{-1} in 50 ml of methanol. This 50 μg ml^{-1} was then used to make serial dilutions of 5, 2.5, 1.5, 1 and 0.5 μg ml^{-1}. The methods are shown in Appendix 2.4. This low range of standards were used as levels at application should not exceed 5 μg ml^{-1} and the range would enable trace concentrations of oxamyl to be detected. The dilutions were injected into the HPLC and a calibration curve produced.

To establish the analytical efficiency soil was obtained from an area near the laboratory where oxamyl had never been applied. Three 20g sub-samples of sieved, air-dried soil were weighed into 100 ml jars. To one jar, 2 ml of the 50 $\mu g\ ml^{-1}$ solution was added to give a soil concentration of 5 $\mu g\ ml^{-1}$, and to another jar 1 ml of 50 $\mu g\ ml^{-1}$ solution was added to give 2.5 $\mu g\ ml^{-1}$. The third jar was left as a control, so that a missing peak in the sample could be identified as the oxamyl peak in the spiked samples. The jars were shaken by hand for 5 minutes, then left for an hour to allow the chemical to equilibrate. The oxamyl was then extracted using the method described, injected into the HPLC and the concentration present established.

2.2.5 Modelling

A model called Persist (Walker, 1974), was used to predict field degradation. It is explained in Walker and Barnes (1981) and further revised by Walker (1987). Data for the model can be found in Appendix 2.5. The basis of the model was to predict the degradation of the chemical by estimating fluctuations in the temperature and moisture content of surface soil from weather records, and to combine these with laboratory measurements of degradation rates. The laboratory data was obtained in Chapter 4.

2.2.6 Statistical analyses

All data were analysed using either analysis of variance or regression analysis procedures using Genstat Version 5.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire).

2.3 RESULTS

The mean concentrations of oxamyl ($\mu g\ g^{-1}$) in the soil with days after application, and the corresponding percentage remaining are shown in Table 2.4. The most rapid degradation was found at the site *Round* with a complete breakdown of oxamyl 21 days after application. At other sites the chemical persisted for around 42 – 56 days with oxamyl still detectable at *Davies* and *Otter* 91 days after application. Fluctuations in the data made it difficult to obtain an accurate Dt_{50}, however at *Bigwood, Stockton, Heaths* and *Round* the time for 50 % disappearance was between 7 –14 days compared with 21 –28 days at all other sites.

The percentage of oxamyl remaining at each site was plotted on a graph, and linear regression analysis used to obtain the *b* (slope) parameter. This figure was then used to obtain an ANOVA of differences between sites (Figure 2.1). *Round* was the only site significantly different to all the others ($P < 0.05$) with the rate of degradation declining much more rapidly. The letters in the chart represent the groups of other sites which were declining at significantly similar rates ($P > 0.05$). Oxamyl was found to be degrading the slowest at *Otter*.

Texture and chemical analyses of the soils displayed similar properties at the 10 sites (Table 2.5). All soils were between a sand and sandy loam texture, the pH range was 5.91 to 7.00, and the organic matter content varied from 2.3 – 3.3 %. The one exception was *Searchlight*, which had a higher organic matter content of 5.3 % due to a grass ley having being grown in the previous year.

Days	Bigwood		Stockton		Heaths		Round		Common	
0	0.88	100	2.17	100	3.51	100	1.14	100	0.80	100
7	0.59	67.05	1.03	47.47	1.97	56.01	0.67	58.42	0.37	45.88
14	0.46	51.93	0.62	28.57	1.24	35.38	0.51	45.09	0.45	56.25
21	0.16	18.18	0.93	42.86	0.55	15.58	0.03	2.63	0.48	60.00
28	0.00	0.00	0.49	22.72	1.17	33.45	0.00	0.00	0.44	55.38
35	0.19	21.93	0.46	21.20	0.38	10.91	0.00	0.00	0.31	38.88
42	0.20	22.73	0.27	12.30	0.32	9.12	0.00	0.00	0.09	10.75
49	0.27	30.68	0.00	0.00	0.72	20.51	*	*	0.11	13.75
56	0.14	15.91	0.00	0.00	1.45	41.31	*	*	0.00	0.00
63	0.23	26.14	0.00	0.00	0.44	12.54	*	*	0.00	0.00
70	0.00	0.00	*	*	0.17	4.84	*	*	0.00	0.00
77	0.02	2.27	*	*	0.16	4.56	*	*	*	*
84	0.00	0.00	*	*	0.00	0.00	*	*	*	*
91	0.00	0.00	*	*	0.02	0.57	*	*	*	*

Days	Searchlight		Front		Crab		Malt		Otter	
0	1.03	100	0.75	100	2.25	100	1.00	100	1.93	100
7	0.58	56.31	0.57	76.00	2.15	95.69	0.89	88.80	1.02	52.85
14	0.35	33.98	0.50	66.67	1.27	56.27	0.70	69.80	0.57	29.33
21	0.59	57.28	0.40	53.07	1.18	52.27	0.54	54.30	0.55	28.45
28	0.19	18.74	0.32	42.67	0.46	20.44	0.26	25.80	0.70	36.06
35	0.26	25.24	0.50	66.27	0.39	17.33	0.15	15.00	0.75	38.86
42	0.00	0.00	0.22	29.33	0.56	24.71	0.00	0.00	0.33	17.31
49	0.14	13.88	0.24	31.60	0.07	3.11	0.06	6.00	0.53	27.56
56	0.16	15.53	0.13	16.93	0.10	4.53	0.12	12.00	0.28	14.72
63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	16.58
70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	11.92
77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	4.66
84	*	*	*	*	*	*	*	*	0.02	1.04
91	*	*	*	*	*	*	*	*	0.05	2.59

Table 2.4 *Oxamyl concentrations in the soil with days after application (ppm values and* ***percentage remaining).***
* no further analysis made

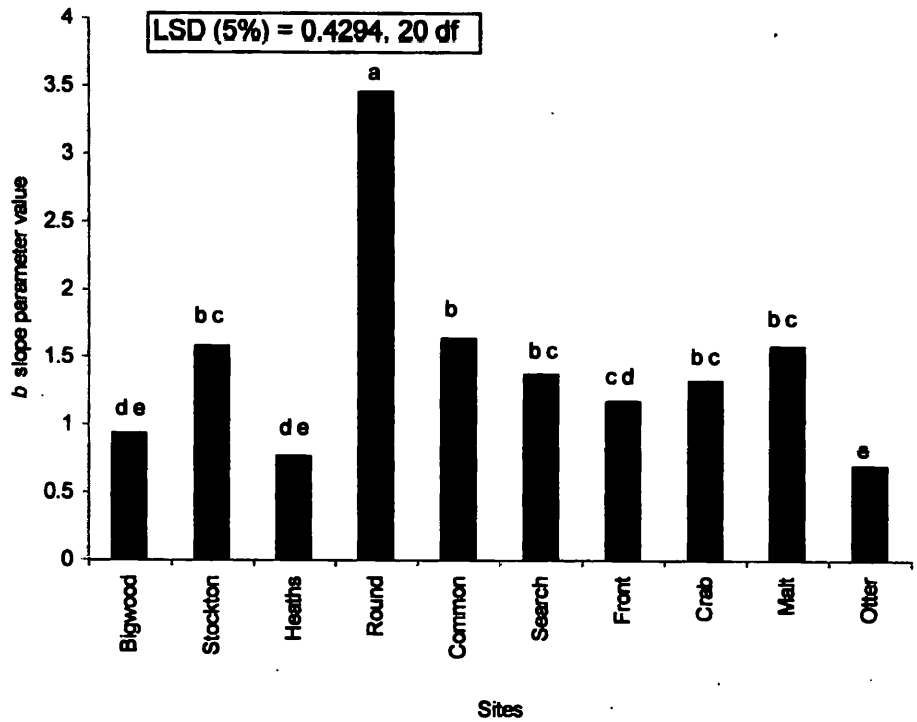


Figure 2.1 *The analysis of variance of sites based on differences in the b* slope parameter which was obtained by linear regression

The letters represent the groups which were not significantly different (P > 0.05)
The data was obtained by the LSD

The ANOVA table belows shows that sites were significantly different from each other

Source	DF	Mean Square	F-value	Probability
Site	9	2.44	28.8	<.001
Residual	20(7)	0.08		

Site	%							
	Sand			clay	silt	textural class	pH	organic matter
	coarse	medium	fine					
Bigwood	5.44	43.74	35.68	3.37	11.19	loamy sand	6.61	3.27
Stockton	8.34	48.93	34.28	3.38	4.48	sand	6.56	2.89
Heaths	4.67	49.24	31.76	3.85	9.89	sand/loamy sand	6.61	2.33
Round	3.82	49.55	34.55	5.19	13.17	loamy sand	7	2.48
Common	5.06	50.9	29	4.29	10.14	loamy sand	6.39	3.04
Searchlight	7.26	46.2	27.52	5.19	13.17	loamy sand	6.68	5.35
Front	3.69	48.19	30.27	7	10.09	loamy sand	6.47	3.89
Crab	4.41	41.51	34.4	5.77	13.22	loamy sand	5.91	3.39
Malt	3.05	59.65	29.79	3.85	3.07	sand	6.3	2.42
Otter	4.31	52.07	33.1	0.72	9.39	sand/loamy sand	6.48	2.71

Table 2.5 *Soil properties at each site*

Data for the simulated decline of oxamyl obtained from the Persist model are shown in Table 2.6. The simulated decline was compared with data from the field using exponential (sympotic regression), (Figures 2.2 *a* – *j*). With all sites 85 to 96 % of the variation was accounted for when separate *a*, *b*, and where applicable *d* parameters were used (see Appendix 2.6), the *c* parameter was a constant. Results showed an insignificant difference between the modelled and field data at *Common, Searchlight, Front* and *Crab* suggesting that a successful prediction of the degradation of the chemical in the field had been made. At all other sites the modelled data was significantly different to the field.

The average soil temperatures over 7 day intervals and the cumulative temperature in day degrees are shown in Tables 2.7 and 2.8. Unfortunately the data loggers failed at *Malt* and *Bigwood* and so these sites could not be included in the analyses. The accumulation of heat related to the time of planting with the later planted site of *Crab*, (planted 8th May) acquiring day degrees faster than sites such as *Otter* and *Davies* which were planted in March. The parameters for multiple regression analysis of oxamyl degradation are shown in Table 2.9. The impact of the average soil temperature, cumulative temperature and rainfall were assessed.

At all 10 sites temperature of the soil, either cumulative or averaged was found to have a more significant impact on oxamyl degradation than rainfall. The relationship was particularly strong at *Round, Searchlight*, and *Common* where average temperatures accounted for 60 – 72 % of the variation, and at *Crab* where cumulative temperature accounted for 62 %. Rainfall only added 2-19 % of the cumulative variation. At other sites where a weaker relationship to temperature was found ($R^2 = < 0.43$), additional factors, which were not measured, were probably having a greater impact on degradation.

Days	Bigwood		Stockton		Heaths		Round		Common	
0	99.94	**100**	99.95	**100**	100	**100**	100.00	**100**	99.91	**100**
7	82.34	**67.05**	86.45	**47.47**	90.96	**56.01**	83.06	**58.42**	77.17	**45.88**
14	76.17	**51.93**	81.57	**28.57**	79.53	**35.38**	73.42	**45.09**	67.62	**56.25**
21	66.02	**18.18**	73.16	**42.86**	68.9	**15.58**	64.26	**2.63**	57.82	**60.00**
28	52.72	**0.00**	61.37	**22.72**	64.84	**33.45**	54.86	**0.00**	41.50	**55.38**
35	46.59	**21.93**	55.68	**21.20**	57.07	**10.91**	48.39	**0.00**	34.82	**38.88**
42	38.91	**22.73**	48.81	**12.30**	48.18	**9.12**	40.42	**0.00**	27.40	**10.75**
49	32.39	**30.68**	42.37	**0.00**	43.51	**20.51**	31.56	*	21.16	**13.75**
56	28.00	**15.91**	38.18	**0.00**	37.5	**41.31**	25.12	*	17.16	**0.00**
63	21.74	**26.14**	31.63	**0.00**	32.66	**12.54**	20.01	*	12.28	**0.00**
70	15.88	**0.00**	24.93	*	28.89	**4.84**	16.01	*	8.02	**0.00**
77	12.47	**2.27**	20.69	*	23.5	**4.56**	13.90	*	5.73	*
84	9.66	**0.00**	16.96	*	18.32	**0.00**	11.37	*	3.92	*
91	0.00	**0.00**	13.95	*	14.86	**0.57**	9.27	*	2.74	*

Days	Searchlight		Front		Crab		Malt		Otter	
0	99.79	**100**	99.91	**100**	99.79	**100**	99.94	**100**	99.94	**100**
7	66.92	**56.31**	76.10	**76.00**	63.27	**95.69**	88.67	**88.80**	93.86	**52.85**
14	51.48	**33.98**	68.24	**66.67**	45.93	**56.27**	76.06	**69.80**	80.55	**29.33**
21	42.15	**57.28**	55.66	**53.07**	25.86	**52.27**	71.51	**54.30**	68.95	**28.45**
28	25.51	**18.74**	40.11	**42.67**	12.50	**20.44**	63.83	**25.80**	63.24	**36.06**
35	17.62	**25.24**	33.47	**66.27**	7.02	**17.33**	53.36	**15.00**	57.89	**38.86**
42	12.37	**0.00**	26.13	**29.33**	3.86	**24.71**	48.36	**0.00**	47.56	**17.31**
49	8.13	**13.88**	20.06	**31.60**	2.13	**3.11**	41.94	**6.00**	42.06	**27.56**
56	5.81	**15.53**	16.48	**16.93**	1.44	**4.53**	36.25	**12.00**	36.73	**14.72**
63	3.87	**0.00**	11.57	**0.00**	0.77	**0.00**	32.32	**0.00**	31.31	**16.58**
70	1.94	**0.00**	7.42	**0.00**	0.41	**0.00**	26.44	**0.00**	27.57	**11.92**
77	0.98	**0.00**	5.23	**0.00**	0.24	**0.00**	20.62	**0.00**	22.94	**4.66**
84	0.54	*	3.61	*	*	*	17.01	*	17.87	**1.04**
91	0.30	*	2.51	*	*	*	13.88	*	14.21	**2.59**

Table 2.6 Simulated data of the percentage of oxamyl remaining using the Persist model compared with actual field concentrations of oxamyl with days after application * (field data is in Bold).

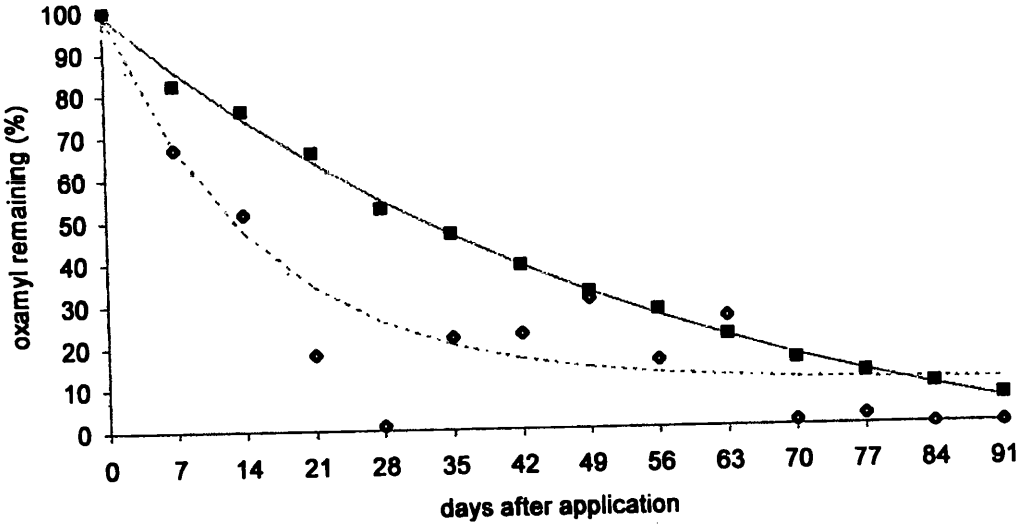


Figure *a* Bigwood
modelled data (■) = (y = -21.4+ 120.2* 0.98381^{x})
field data (◊) = (y = 10.19 + 88.58* 0.9398^{x})
modelled data significantly different from field data (P< 0.05)

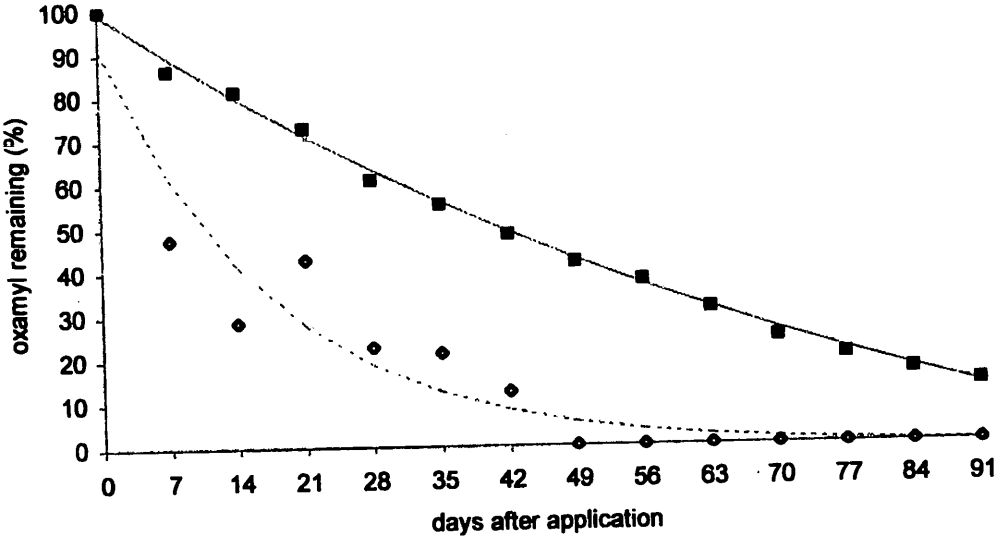


Figure *b* Stockton
modelled data (■) = (y = -38 + 137.2*0.98921^{x})
field data (◊) = (y = -0.48 + 91.57* 0.94565^{x})
modelled data significantly different from field data (P< 0.05)

Figure 2.2 a-j *The mean percentage of oxamyl remaining in the field, compared with data predicted using a Persist model*

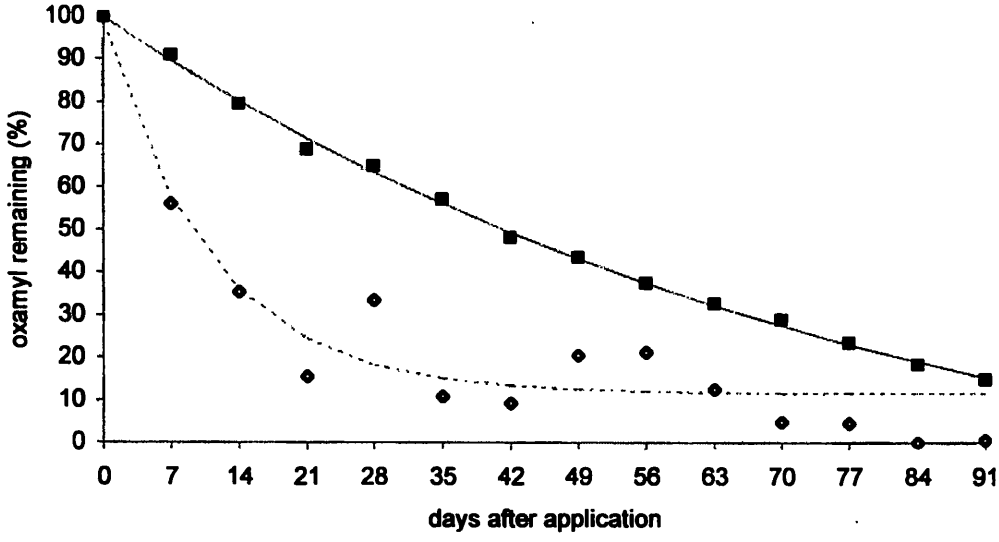


Figure *c*. Heaths
modelled data (■) = ($y = -26.4 + 126.3 * 0.98791^x$)
field data (◊) = ($y = 11.5 + 87.32 * 0.9128^x$)
modelled data significantly different from field data ($P < 0.05$)

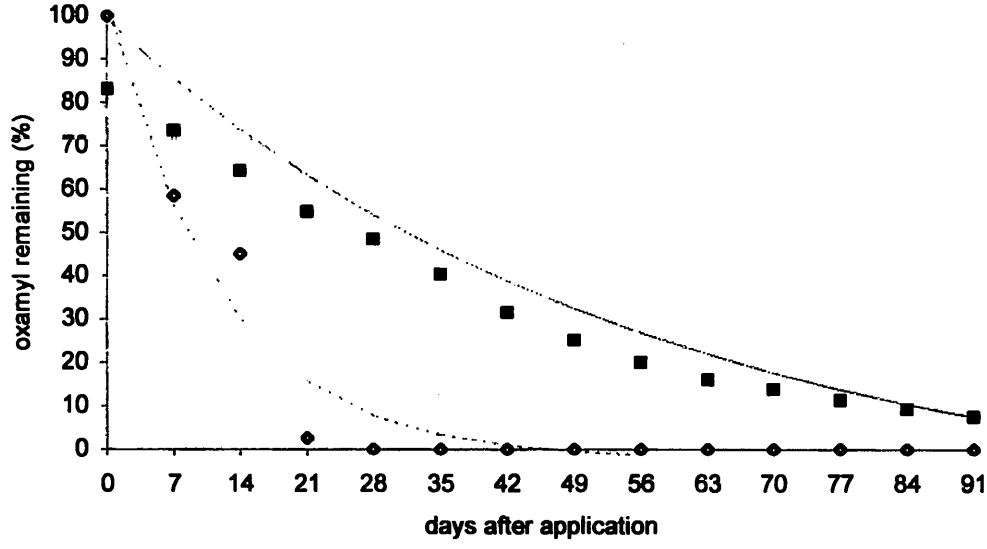


Figure d. Round
modelled data (■) = ($y = -14.7 + 113.7 * 0.98222^x$)
field data (◊) = ($y = -2.07 + 104.66 * 0.91923^x$)
modelled data significantly different from field data ($P < 0.05$)

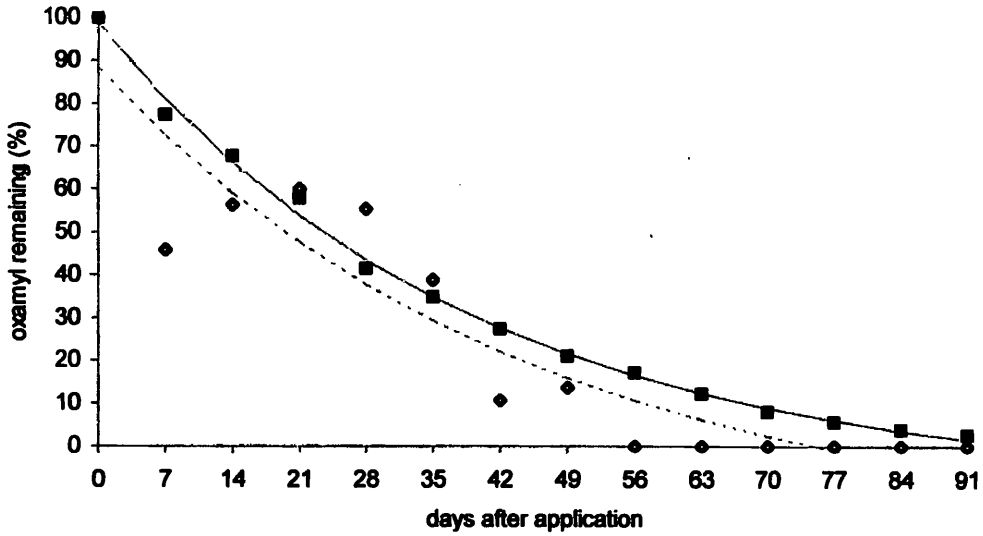


Figure e Common
modelled data (■) ($y = -8.6 + 107.3 * 0.97449^x$)
field data (◊) = ($y = -20.3 + 208.4 * 0.97794^x$)
modelled data is not significantly different from field data (P> 0.05)

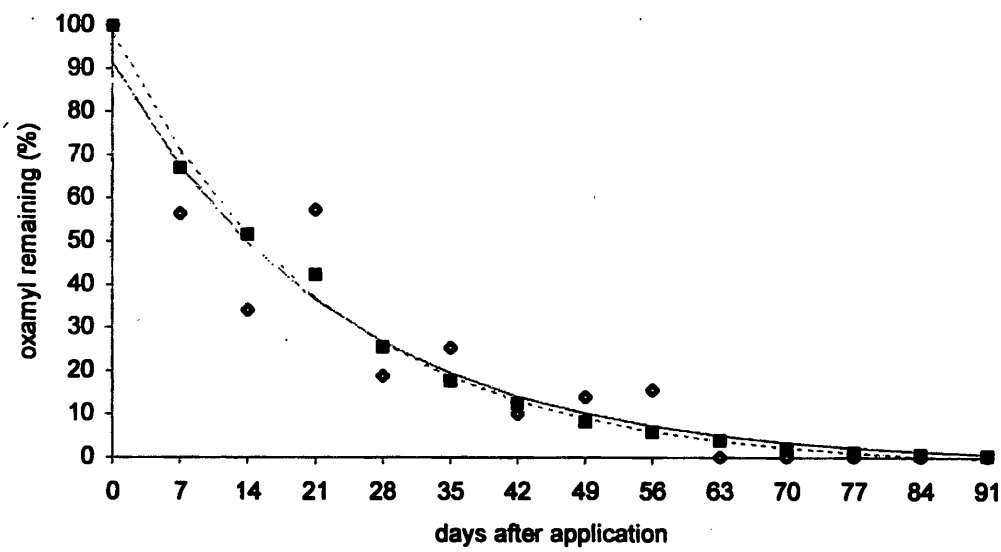


Fig. *f*. Searchlight
modelled data (■) = ($y = -2.07 + 100.29 * 0.95596^x$)
field data (◊) = ($y = -1.11 + 92.39 * 0.95798^x$)
modelled data is not significantly different from field data (P> 0.05)

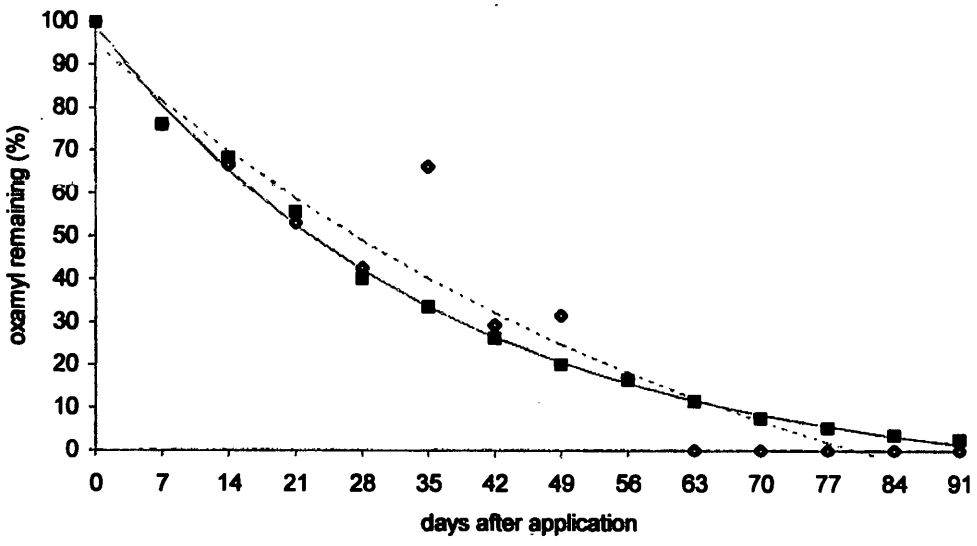


Figure *g* Front
modelled data (■) = (y = -45.3 +140.1*0.986^{x})
field data (◊) = (y = -7.5+ 106.32*0.97321^{x})
modelled data is not significantly different from field data (P> 0.05)

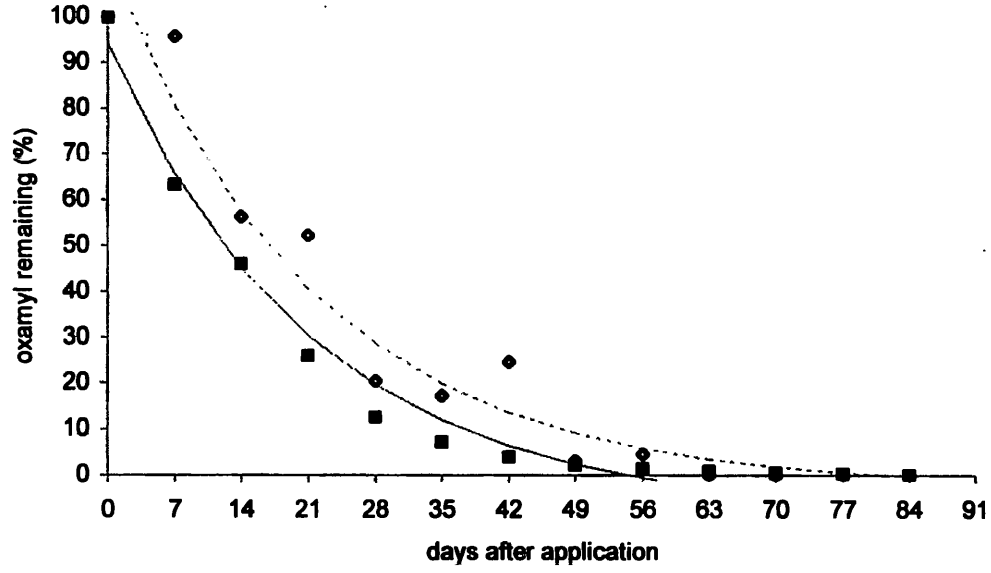


Figure *h* Crab
modelled data (■) = (y = -2.6 + 115.2* 0.95^{x})
field data (◊) = (y = -8.04 + 102.2 * 0.95^{x})
modelled data is not significantly different from field data (P> 0.05)

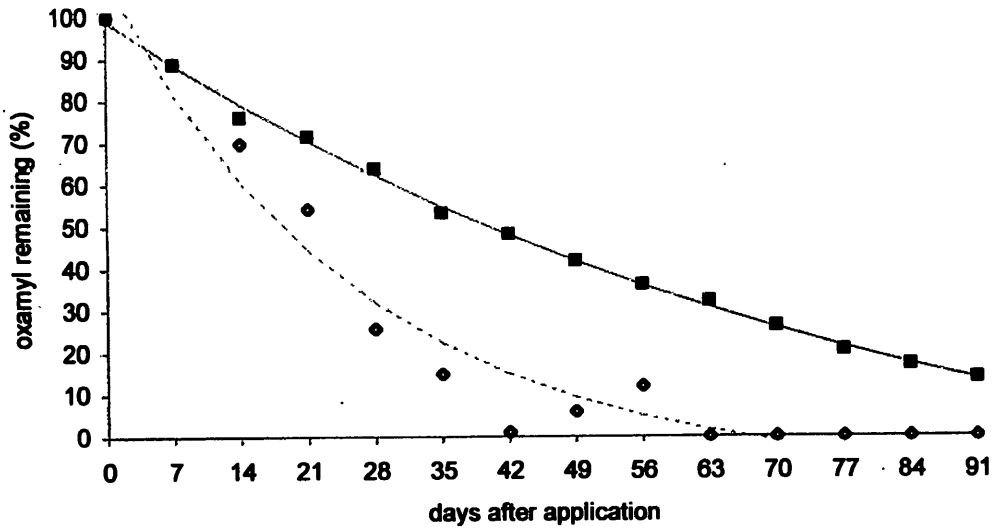


Figure *i* Marsh
modelled data (■) = (y = -29.7 + 128.6* 0.98812^{x})
field data (◊) = (y = -9.71 + 118.59* 0.96341 ^{x})
modelled data significantly different from field data (P< 0.05)

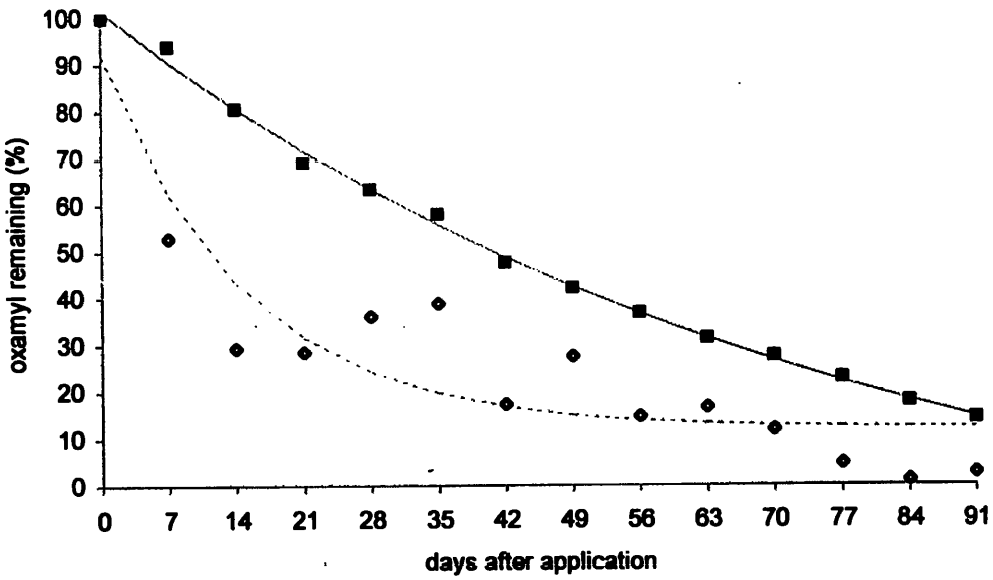


Figure *j* Otter
modelled data (■) = (y = -23.8 + 125.3* 0.98709^{x})
field data (◊) = (y = 12.05 + 80.01 * 0.9351 ^{x})
modelled data significantly different from field data (P< 0.05)

Days	Sites							
	Stockton	Heaths	Round	Common	Search	Front	Crab	Otter
7	8.6	7.9	10.4	9.0	9.5	8.8	18.2	8.0
14	4.9	10.4	11.2	5.7	6.6	5.2	15.4	10.7
21	8.0	8.6	13.8	7.4	6.4	7.9	15.8	8.7
28	10.8	5.0	18.6	10.7	9.8	11.2	14.3	5.4
35	11.8	7.8	15.1	11.7	11.6	12.1	14.0	8.0
42	14.5	10.4	15.3	14.1	13.2	15.1	16.0	9.7
49	18.4	11.5	14.6	18.6	16.8	19.4	14.5	12.2
56	13.9	14.4	13.0	16.0	17.0	15.2	15.1	14.6
63	15.0	18.7	15.9	15.2	14.8	15.8	14.5	18.2
70	13.9	14.2	14.3	14.4	14.7	14.5	14.8	13.9
77	12.7	14.6	14.5	12.9	13.8	13.2	14.8	13.8
84	15.5	14.5	14.3	15.5	15.5	16.0	14.9	12.8

Table 2.7 *The average weekly soil temperatures calculated from the maximum and minimum values per day*

Days	Sites							
	Stockton	Heaths	Round	Common	Search	Front	Crab	Otter
7	35.6	31.1	44.0	38.7	42.0	34.9	98.6	27.1
14	46.4	79.7	93.7	54.2	63.8	45.6	177.5	73.3
21	77.7	115.4	161.6	81.3	84.0	74.0	259.4	105.6
28	128.6	126.5	263.0	131.6	128.1	126.1	330.8	116.8
35	186.8	156.5	339.7	188.7	184.9	183.9	400.1	144.4
42	263.5	204.7	418.4	264.9	252.6	263.2	483.7	194.7
49	368.1	261.0	492.1	370.3	345.9	372.7	556.3	251.6
56	440.6	337.0	554.5	457.7	440.4	452.4	633.4	325.4
63	521.3	443.4	636.9	539.8	519.7	536.1	706.0	424.4
70	594.3	518.4	708.3	616.2	598.4	611.3	780.9	493.2
77	658.7	596.1	781.3	681.7	670.3	677.0	855.6	561.3
84	746.7	673.0	852.8	765.6	752.8	762.6	931.3	628.4

Table 2.8 *The cumulative temperature in day degrees C.*
Assumed basal temperatures = 4.1 °C for G. rostochiensis and 3.5 °C for G. pallida

Site	a	b	F-value	Probability	R^2
Stockton					
Cumulative temp.	0.1 (0.42)	0.002 (0.0007)	8.15	0.006	0.37
Rainfall		0.022 (0.02)	1.18	0.28	0.4
Heaths					
Average temp.	3.18 (0.27)	0.02 (-7.81)	35.91	<.001	0.42
Rainfall		0.01 (-4.88)	23.81	<.001	0.61
Round					
Average temp.	1.09 (0.76)	- 0.06 (0.006)	127.3	< .001	0.72
Cumulative temp.		- 0.0004 (0.0001)	16.16	< .001	0.79
Common					
Average temp.	0.84 (0.08)	-0.05 (0.005)	77.92	<0.001	0.6
Rainfall		-0.004 (0.003)	1.9	0.17	0.62
Searchlight					
Average temp.	1.01(0.07)	- 0.06 (0.05)	105.7	<0.001	0.68
Rainfall		-0.007 (0.002)	8.27	0.005	0.73
Front					
Cumulative temp.	0.74 (0.12)	-0.0007 (0.0002)	37.91	< .001	0.43
Average temp.		- 0.016 (0.01)	1.74	0.19	0.02
Rainfall		-0.005 (0.004)	1.57	0.22	0.02
Crab					
Cumulative temp.	1.33 (0.22)	-0.002 (0.0002)	80.39	<.001	0.62
Rainfall		-0.02 (0.009)	4.63	0.04	0.65
Average temp.		0.04 (0.02)	5.22	0.03	0.68
Otter					
Average temp.	1.21 (0.18)	-0.06 (0.02)	14.78	<.001	0.23

Table 2.9. Parameters (± standard error) for multiple regression analysis of the impact of the weekly average soil temperature, cumulative temperature and rainfall on oxamyl *(y)* degradation

The factor of most significance is listed first. If highly insignificant the factor was ommitted from the analysis

Malt and Bigwood were not included as temperature data was not available

2.4 DISCUSSION

The more rapid degradation of oxamyl at *Round* (Fig. 2.1) compared to at all other sites would suggest that enhanced rates of breakdown were occurring. At *Round* average and cumulative soil temperatures had a strong relationship with oxamyl degradation accounting for 79 % of the variation. Rainfall was not a significant factor whereas at all other sites, apart from *Otter* rainfall accounted for about 5 % of the variation. Interestingly, *Otter,* which was found to be the slowest degrading site, had the weakest relationship with temperature ($R^2 = 0.23$).

However if there were a relationship between degradation and temperature, the breakdown of oxamyl at *Crab*, which was the last site to be planted, would have been expected to be fastest. Data shows that 7 days after oxamyl incorporation, the average soil temperature at *Crab* was approximately 18 °C whereas at *Round* it was 10 °C. The cumulative temperature at *Crab* was also always distinctly higher than at all other sites throughout the growing season. An affinity to temperature has been demonstrated by Smelt *et al.* (1978) who found that in soils incubated at 6, 15 and 25 °C, higher temperatures greatly increased the rate of transformation of Aldicarb Sulphoxide. Gerstl (1984) also found the degradation of oxamyl proceeded more rapidly at higher temperatures. However Jukes *et al.* (1996) found that in a sandy loam, carbofuran degradation rates increased with increasing moisture and temperature and so the combination of high moisture levels and high soil temperatures at *Round* could explain why the oxamyl degraded much more rapidly than at *Crab*.

Figure 2.3 shows the monthly rainfall and planting dates of the different sites. Within 7 days of planting, 24.8 mm rainfall had fallen at *Front, Common* and *Searchlight*, 14.8 mm at *Otter* and *Heaths*, 17.0 mm at *Round* and 4.5 mm at *Crab*. At *Crab* the soil was not irrigated until July. This factor along with the absence of rainfall for much of May would have resulted in a poor distribution of the chemical to depths below the level of incorporation. Smelt *et al.* (1979) found that decreasing the moisture content of the soil to about wilting point slowed the conversion of oxamyl and this would appear to be the case at *Crab* as between 0 –7 days after application only 5 % of the oxamyl had been degraded (Table 2.1).

The rapid degradation at *Round* could have been further enhanced by the high pH of 7.0 compared with *Crab* of pH 5.9 (Table 2.5). Smelt *et al.*, (1979) found the half-life of oxamyl in soils of pH 7.1 - 7.4 was 13-14 days compared with 34-39 days in soils of pH 5.2-5.4. This was due to the increased hydrolysis in alkaline soils. The organic matter content at *Round* was also lower than at *Crab* resulting in slightly less adsorption and greater mobility (Smelt and Leistra, 1992).

The low impact of rainfall on degradation could be associated with the overall sandy textures of the soils. These would have had a low water holding capacity and so the chemical would have been readily leached through the profile (Whitehead *et al.*, 1981). Rainfall may have had a greater impact on degradation in clay soils.

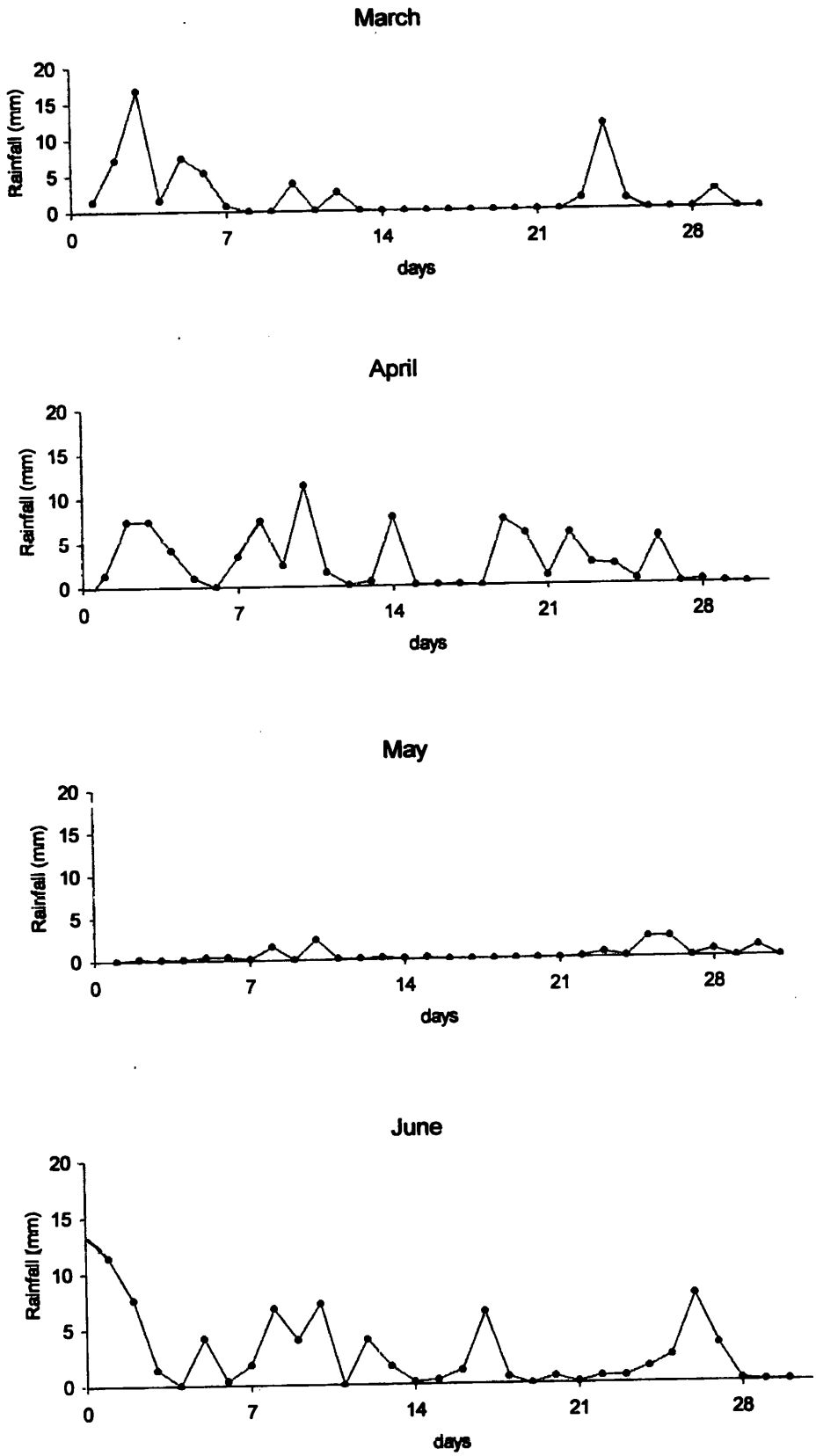


Fig.2.3. *Monthly rainfall data (mm)*
Otter, Heaths, Malt - planted 17-19 March
Searchlight, Common, Stockton, Front & Bigwood - 30 Mar - 2 April
Round - 22 April Crab - 8 May

Modelled data

Simulations by the Persist model showed that at *Common, Searchlight, Crab*, and *Front* the field degradation rates and predicted decline were significantly similar ($P > 0.05$). At all other sites degradation in the field was occurring at a more rapid rate than predicted. This would suggest that certain factors were having an impact on one group of soils and not on the other.

Temperature and rainfall were unlikely to be the cause of these differences as *Stockton* and *Bigwood*, where the field and simulated decline were different, were planted in the same week as *Searchlight, Common* and *Front*. As the sites were within a 15 mile radius they would have experienced similar rainfall and soil temperatures. Differences were also unlikely to be due to incorporation techniques as *Crab* and *Davies*, and *Front* and *Otter*, were owned by the same growers and so application techniques would have been similar.

One possibility could be the texture of the soil. The Persist model was developed for herbicides and only predicts the soil water content and soil temperature in the top 50 mm. As a result leaching to deeper soils is not accounted for. Soil texture analysis, (Table 2.5) showed that sites where simulated and field decline were similar were all loamy sands (apart from *Round*) with relatively high organic matter contents (OM). The others were of a more sandy texture with a lower OM. At these latter sites the oxamyl would have been less weakly sorbed during incorporation and as a result it could have been leached more through the profile.

Another explanation could be that the groups of sites contained different microbial degrading populations. This theory will be further studied in Chapter 4.

2.5 CONCLUSIONS

- Monitoring of 10 treated fields in Shropshire revealed wide variations in nematicide persistence, with the time taken for half of the oxamyl to disappear from the soil ranging from 10 to 24 days.

- The degradation of oxamyl was significantly faster at one site (*Round*) suggesting that enhanced degradation could be occurring. At all sites the average or cumulative soil temperatures had a more significant impact on oxamyl degradation than rainfall. However, it is probable that the rapid breakdown at *Round* was the result of a combination of warm temperatures throughout the growing season combined with moist soils and high pH.

- The Persist model predicted the same rate of decline of oxamyl as occurred in the field at only four out of the ten sites. At the other sites degradation in the field was occurring at more rapid rates. This could be as a result of the Persist model not accounting for the movement of nematicides by leaching, or it could be because enhanced degradation of nematicides was occurring at these latter sites, or it could be a combination of both these factors.

Chapter 3

The degradation of oxamyl and subsequent invasion of roots by potato cyst nematodes

3.1 INTRODUCTION

The effectiveness of granular nematicides depends on the carrier granules' distribution in the soil and the ability of the active ingredient to reach the nematodes in adequate concentrations for a certain minimum period of time. After this, conversion to non-toxic oximes needs to occur to prevent residues and leaching in to ground water.

In vitro studies have been made into the toxic concentrations required to control nematodes. Evans and Wright (1982) found that exposure to 2.0 μg ml $^{-1}$ oxamyl for four hours caused the motility of G. rostochiensis juveniles to be directly affected, with spasmodic movements becoming apparent. Similar behaviour was observed after 24 h at 0.2 μg ml $^{-1}$ oxamyl. Hague and Pain (1973) found that control was achieved at 5μg g^{-1} aldicarb, however after 6 weeks when the 5μg g^{-1} concentration had declined to non-inhibitory levels, nearly 90 % of the eggs had hatched.

However these studies were carried out *in vitro* and such conditions may not occur in the field. Evans and Wright (1982) and Osbourne (1973) who studied aldicarb, used diffusate and nematicide as the only media. The experiments were carried out on watchglasses or screw cap vials, and conditions were held at constant temperatures of 20 or 24 °C. As a result, toxic concentrations found to control nematodes in the laboratory may not have been an accurate representation of the levels of control achieved in the field.

Jones and Parrot (1969) suggested that nematode invasion and development was controlled by temperature measured in accumulated day degrees above an assumed basal temperature. Evans (1968) found that in a sandy soil, with a basal temperature of 4.4°C, the development from one stage to the next occurred after a certain number of day degrees had accumulated.

Objectives

- To investigate the rates of degradation of oxamyl in relation to nematode hatching characteristics in field situations.

- To estimate the minimum concentration of nematicide necessary in soil to prevent invasion.

- To establish whether *G. pallida* is as effectively controlled using granular nematicides as *G. rostochiensis*.

- To provide evidence to support the claim that nematode development is controlled by cumulative temperature measured in day degrees.

3.2 MATERIALS AND METHODS

3.2.1 Nematode population density assessments

Sampling in the field

To ascertain the levels of control achieved at each site nematode densities were determined prior to planting and after harvest. Fields were sampled in a systematic grid pattern by walking up and down the areas with the highest estimated population densities. This data was obtained from the growers (Section 2.2.1). A total of fifty cores were taken, one every fifty paces, using a cheese-type auger to a depth of 300mm x 25mm. The cores were bulked together in a bucket, mixed using a hand trowel, then divided into a porous sampling bag and a polypropylene bag. The soil in the polypropylene bag was frozen at – 14 °C for future analyses.

Preparation of soil samples

The soils were fully air-dried by storing the unopened sample bags in a forced air-flow drying cupboard at 25 °C for one week. They were then sieved through a 4 mm aperture, mixed thoroughly and a 200 gram sub-sample was measured into a plastic container and labelled.

Extraction of the cysts from the soil

The cysts were extracted using the Fenwick Can (Southey, 1940). The soils were washed through a 1mm sieve, followed by an 841µm and then a 250µm sieve. The cysts were washed from the final sieve, using water from a wash bottle, onto a piece of dampened fine

mesh linen (approximately 20 x 20 cm). The linen was folded to secure the cysts and fastened with a paper clip. The linen was then dried in the air cupboard for 24 hours.

Counting and opening the cysts

The dried cysts were poured through a small funnel into a collecting tube. This was then sprinkled onto a counting tray, placed under a stereo microscope, and examined at 30x magnification. All *Globodera* cysts in the sample were counted. The first fifty (or number present if less than 50) were extracted with tweezers, transferred to a watch glass, and soaked in 2 ml of sterile distilled water for 24 hours at room temperature.

Using a pipette, the water was transferred from the watch glass into a graduated boiling tube. The cysts were then removed, using tweezers, into a channelled aluminium slide (0.05 mm deep central channel). They were gently squashed under a glass slide then carefully washed into the boiling tube. If 50 cysts were present the boiling tube was filled to 50 ml with distilled water, and if less than 50 cysts, to 25ml.

Counting the eggs and juveniles

Using a 5ml pipette the suspension was agitated for 30 seconds to separate the eggs and homogenise the solution. A pipette of the sample was then collected and transferred to a counting slide, holding 1ml of solution. The slide was placed under a stereo microscope (x50 magnification) and the number of eggs and juveniles counted in the grid. Calculations for the number of juveniles per gram are shown in Appendix 3.1.

3.2.2 Nematode species determination

The proportion of species in each site was determined by the Isoelectric Focusing (IEF) of the nematode proteins (Fleming and Marks, 1983).

3.2.3 Field Sampling

Plant samples were taken on the week of planting and then at weekly intervals for 13 weeks. A single plant sample was lifted from each station with a spade. The ridges were severed on both sides of the potato at equal distances from the neighbouring plants, the soil was loosened and the plant lifted out with a fork. When the leaves developed, about 7 weeks after planting, the lower stem and root system were removed by cutting the stem at the point that it became green. The root system was placed into a labelled polypropylene bag and the soil forked through to collect any loose roots.

The rate of potato growth was assessed by recording the date that the potato shoots reached the soil surface. The accumulated temperature in day degrees was obtained from the data loggers described in section 2.2.3.

3.2.4 Quantification and identification of nematodes in roots

Preserving the roots

The roots were washed to remove stones and soil. They were then removed from the main stem, and cut into 2 cm lengths. These were mixed and a 2 g sub-sample weighed into a 30 cm^3 glass specimen tube. Sufficient formal acetic alcohol preservative (Hooper, 1986) was added until the roots were completely covered. The specimen tubes were labelled and stored at room temperature prior to staining.

Staining nematodes in the roots

In a fume cupboard the roots were emptied on to a fine mesh (10 x 10 cm), and the sampling tube rinsed. The corners of the mesh were gathered to form a loose bundle and secured with the end of a wire sample holder (Woods, *pers. com.*). To stain the nematodes, 60 ml of acid-fuchsin solution (Bridge *et al.*, 1982) was poured into a glass beaker (1 litre) and heated on a hot-plate. Once boiling, the roots, in bundles of six, were gently lowered into the stain solution for 3 minutes and 30 seconds. To clear excess stain the roots were briefly agitated in water (1 litre).

Quantitative assessment of nematode developmental stages

The mesh was unwrapped and the roots cut into shorter lengths. These were placed into a Waring commercial blender and distilled water added to cover the uppermost blades of the jug (approximately 150 ml water). The blender was run on a low and then high setting, each for 30 seconds. The roots and water were then transferred to a graduated 400 ml beaker and made up to 200 ml with distilled water. Using a 5 ml pipette the solution was agitated and then 5 ml of solution withdrawn. From the pipette, 1 ml was passed back into the beaker and 2 ml transferred to a De Grisse counting tray from which the nematode stages were recorded using a stereo microscope (x 50 magnification).

3.2.5 Statistical analyses

Multiple regression analyses on invasion and development of the nematode was carried out using Genstat Version 5.1.

3.3 RESULTS

Figure 3.1 shows the first detected invasion of PCN in potato roots, in relation to the time of oxamyl application. At *Bigwood, Heaths, Searchlight* and *Common* no invasion was observed until 35-42 days after nematicide incorporation, however at all other sites entry into the plant was first detected after 14-21 days, and 7 days at *Crab*.

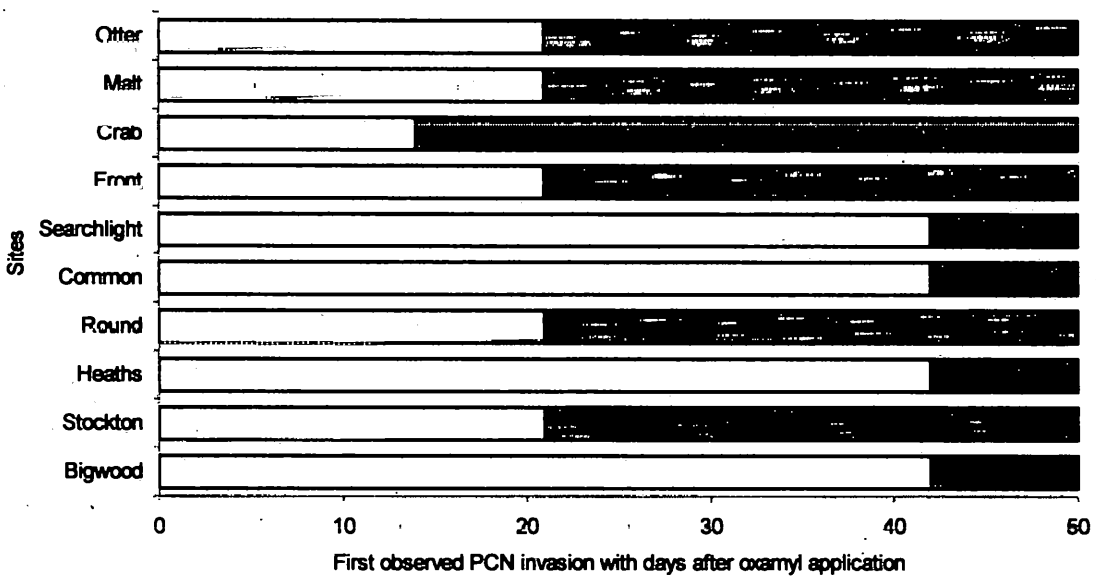


Figure 3.1 *The shaded areas show the number of days after nematicide application when nematodes were detected in the roots, unshaded means no invasion was detected.*

At *Round* and *Crab* the early invasion resulted in high numbers of juveniles in the roots, with development to J4 stages being apparent 28 days after oxamyl application (Tables 3.1a-j). At *Stockton, Malt* and *Front* juvenile invasion also began early, however development into later stages was not observed until 70 days after planting. This coincided with large numbers of J3 and J4 being present at *Searchlight, Common* and *Heaths*. At *Bigwood* and *Otter* development beyond J3 stages was not observed.

A.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	0	0	0	0	0	0
28	0	0	0	0	0	0
35	0	0	0	0	0	0
42	13	0	0	0	0	0
49	25	0	0	0	0	0
56	13	0	0	0	0	0
63	38	0	0	0	0	0
70	13	13	0	0	0	0
77	0	0	0	0	0	0
84	38	0	0	0	0	0
91	38	0	0	0	0	0

B.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	75	0	0	0	0	0
28	113	0	0	0	0	0
35	50	0	0	0	0	0
42	0	0	0	0	0	0
49	13	13	0	0	0	0
56	25	38	0	0	0	0
63	75	38	13	13	0	0
70	113	288	50	13	38	0
77	38	63	225	0	38	0
84	175	125	238	38	75	0
91	163	38	50	0	25	25

C.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	0	0	0	0	0	0
28	0	0	0	0	0	0
35	0	0	0	0	0	0
42	38	0	0	0	0	0
49	38	38	0	0	0	0
56	38	75	0	0	0	0
63	25	0	0	0	0	0
70	38	0	0	0	0	0
77	175	150	0	0	13	0
84	100	263	50	0	0	0
91	75	38	75	0	38	0

Tables 3.1a-j Invasion and development of PCN in potato roots in relation to days after oxamyl application

A. Bigwood, B. Stockton, C. Heaths (m = male, f = female)

D.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	113	0	0	0	0	0
28	325	63	88	13	13	0
35	425	75	0	0	0	0
42	100	50	13	0	0	0
49	288	363	13	0	0	0
56	313	50	38	0	13	0
63	350	75	113	13	0	0
70	125	0	0	0	13	0
77	100	0	0	0	0	0
84	138	13	13	0	0	0
91	113	13	0	0	0	0

E.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	0	0	0	0	0	0
28	0	0	0	0	0	0
35	0	0	0	0	0	0
42	150	63	0	0	0	0
49	200	125	0	0	0	0
56	125	100	13	13	0	0
63	113	50	25	0	0	0
70	175	350	150	25	0	0
77	0	25	75	0	50	0
84	100	25	38	0	25	50
91	50	13	38	0	13	38

F.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	0	0	0	0	0	0
28	0	0	0	0	0	0
35	0	0	0	0	0	0
42	75	38	0	0	0	0
49	225	150	50	13	0	0
56	75	75	13	13	0	0
63	263	113	25	0	13	0
70	138	425	50	0	25	13
77	150	63	113	0	13	0
84	163	88	125	25	38	13
91	113	75	0	13	13	38

D. Round, E. Common, F. Searchlight (m = male, f = female)

G.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	38	0	0	0	0	0
28	0	0	0	0	0	0
35	13	0	0	0	0	0
42	50	0	0	0	0	0
49	13	0	0	0	0	0
56	0	0	0	0	0	0
63	38	0	0	0	0	0
70	13	63	13	0	0	0
77	25	13	75	0	0	0
84	63	13	0	0	0	0
91	25	13	0	0	0	0

H.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	63	0	0	0	0	0
21	100	50	25	0	0	0
28	88	50	138	0	0	0
35	88	25	0	0	0	0
42	25	25	0	0	0	0
49	0	13	13	0	0	0
56	0	25	13	0	0	0
63	50	0	0	0	0	0
70	0	0	0	0	0	0
77	0	0	0	0	0	0
84	0	0	0	0	0	0
91	0	0	0	0	0	0

I.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	25	0	0	0	0	0
28	25	0	0	0	0	0
35	13	0	0	0	0	0
42	38	0	0	0	0	0
49	63	25	0	0	0	0
56	125	88	13	0	0	0
63	100	13	0	0	25	0
70	25	25	0	13	0	0
77	13	0	13	13	0	0
84	50	13	0	0	0	0

H. Front, I. Crab, J. Malt

(m = male, f = female)

J.

Days	nematodes / g root					
	J2	J3	J4m	J4f	J5m	J5f
14	0	0	0	0	0	0
21	50	0	0	0	0	0
28	75	0	0	0	0	0
35	25	0	0	0	0	0
42	88	0	0	0	0	0
49	38	0	0	0	0	0
56	88	0	0	0	0	0
63	125	0	0	0	0	0
70	0	0	0	0	0	0
77	50	0	0	0	0	0
84	75	0	0	0	0	0
91	50	0	0	0	0	0

J. Otter

Table 3.2 shows the time potato shoots were first observed at the soil surface with days after oxamyl application. Development was most rapid at *Crab* where potato shoots emerged 14 days after oxamyl application, followed by *Round* at 21 days. The slowest development required 42 days before the potato shoots reached the surface.

The initial and final population densities and I.E.F. analysis of species are shown in Table 3.3. The greatest population increase over the growing season occurred at *Front* and *Crab* where the Pi / Pf ratio ranged was 6.6 and 7.4, respectively. Populations at *Bigwood, Davies* and *Marsh* increased by 3.3 to 4.5 - fold. The only control was achieved at *Otter* and *Heath* where the Pf was reduced by the end of the season

The parameters for multiple regression analyses on nematode invasion are shown in Table 3.4. The impact of the average weekly soil temperatures, cumulative temperature, rainfall and oxamyl concentration were assessed. Temperature showed a stronger relationship to invasion than rainfall. At *Stockton* 75 % of the variation was accounted for by average soil temperatures and 6 % by rainfall. At all other sites the relationship with invasion was weaker, with temperature accounting for 30 to 40 % of the cumulative variation at *Heaths, Common* and *Searchlight*, and much less at other sites. At *Crab* all variables were found to be insignificant ($P = 0.18$). At *Round* oxamyl was the most significant variable ($P = 0.004$) however this only accounted for 16 % of the variation.

Regression analyses on the development of PCN with cumulative temperature, are shown in Table 3.5. At all sites, apart from *Crab* and to a lesser extent *Round*, a significant relationship (<0.05) was shown. However a strong relationship ($R^2 = 0.75$) between development and temperature was only displayed at *Stockton* and *Common*.

Site	Date of emergence	days after planting
Bigwood	*	*
Stockton	30 – Apr	28
Heaths	28 – Apr	42
Round	13 – May	21
Common	13 – May	42
Searchlight	12 – May	42
Front	7 – May	35
Crab	22 –May	14
Malt	30 –Mar	42
Otter	28 – Mar	42

Table 3.2 *The number of days after oxamyl application when potato shoots first emerged through the soil*

Site	Pi	Pf	Pf / Pi	population	dominant species
	eggs / g soil	eggs /g soil			
Bigwood	2	10	4.5	mixed	* both present
Stockon	23	18	0.8	pure	*G. pallida*
Heaths	11	38	3.3	pure	*G. pallida*
Round	31	46	1.5	mixed	*G. rostochiensis*
Common	38	78	2.1	pure	*G. pallida*
Searchlight	55	73	1.3	mixed	*G. pallida*
Front	6	38	6.6	mixed	* both present
Crab	13	96	7.4	pure	*G. pallida*
Malt	1	4	3.3	pure	*G. rostochiensis*
Otter	48	20	0.4	pure	*G. rostochiensis*

Table 3.3 *Initial and final population densities of PCN and the Pf / Pi ratio*
* No dominant species

Site	a	b	F-value	Probability	R^2
Stockton					
Average temperature	2.07 (0.13)	-0.12 (0.008)	148.06	<0.001	0.75
Rainfall		-0.06 (0.004)	16.39	<0.001	0.81
Heaths					
Cumulative temperature	0.03 (0.40)	0.005 (0.01)	27.52	<.001	0.36
Average temperature		-0.06 (0.05)	1.16	0.29	0.37
Round					
Oxamyl	4.41 (0.64)	- 4.88 (1.61)	9.19	0.004	0.16
Common					
Average temperature	-0.88(0.71)	- 0.22 (0.05)	25	<0.001	0.33
Rainfall		-0.03 (0.02)	1.62	0.21	0.35
Searchlight					
Cumulative temp	10.65 (8.01)	0.004 (0.002)	23.09	<.001	0.31
Rainfall		-0.05 (0.02)	2.04	0.16	0.027
Oxamyl		-2.14 (1.3)	2.85	0.1	0.037
Front					
Cumulative temperature	0.16 (0.16)	0.0008 (0.000	3.95	0.05	0.07
Crab					
Cumulative temperature	0.88 (0.44)	-0.001 (0.0007	1.87	0.18	0.03
Rainfall		0.04 (0.03)	1.79	0.19	0.07
Otter					
Cumulative temperature	0.14 (0.29)	0.001 (0.0007)	3.38	0.07	0.06
Rainfall		0.02 (0.015)	1.4	0.24	0.09

Table 3.4. Parameters (± standard error) for multiple regression analysis of the impact of the weekly average soil temperature, cumulative temperature, rainfall and oxamyl concentration on nematode invasion

The factor of most significance is listed first. If highly insignificant the factor was ommitted from the analysis

Malt and Bigwood were not included as temperature data was not available

Site	a	b	F-value	Probability	R^2
Stockton	0.66 (0.1)	0.003 (0.0002)	142.4	<.001	0.74
Heaths	0.81 (0.1)	0.002 (0.0003)	40.37	<.001	0.45
Round	1.24 (0.19)	- 0.0007 (0.0004)	3.58	0.06	0.07
Common	0.83 (0.13)	0.003 (.0003)	69.68	<.001	0.76
Searchlight	0.77 (0.1)	0.003 (.0003)	*	<.001	*
Front	0.88 (0.1)	0.0009 (0.0002)	13.4	<.001	0.21
Crab	1.11 (0.17)	-0.0005 (0.0003)	2.73	0.1	0.04
Otter	1.05 (0.11)	0.0009 (0.0003)	6.11	0.02	0.11

Table 3.5. Parameters (± standard error) for multiple regression analysis of the impact of cumulative temperature on nematode development

The factor of most significance is listed first. If highly insignificant the factor was ommitted from the analysis, * = unobtained data

Malt and Bigwood were not included as temperature data was not available

3.4 DISCUSSION

With the sites where invasion occurred 42 days after application it could have been suggested that control by oxamyl had been achieved. However regression analyses showed no significant relationship between invasion and oxamyl apart from at *Round* ($P = 0.004$). This is emphasised by the data in Table 3.6, which shows no obvious correlation between the sites where later invasion occurred and higher initial concentrations of oxamyl in the soil. At 14 days after application, concentrations of oxamyl at *Otter, Malt, Stockton* and *Front* ranged from 0.5 to 0.7 ppm. However at *Searchlight* and *Common* where invasion was not observed until 42 days, the oxamyl concentrations at 14 days were 0.45 and 0.35 ppm respectively. Due to fluctuations in oxamyl concentrations at each site and variations between sites, it was impossible to estimate a minimum concentration necessary in the soil to prevent invasion.

Temperature did not have as high an impact on invasion as it did on oxamyl degradation, with the only strong relationship at *Stockton* ($R^2 = 0.75$). At *Front, Crab* and *Otter* less than 6 % of the variation was accounted for by soil temperature, rainfall or oxamyl. As a result it was likely that other factors were responsible for the differences in invasion. One possibility was the rate of development of the potato plant, and subsequent root growth. Photographs of the tubers were taken 5 weeks after planting (Figure 3.2). At *Crab* and *Round,* where invasion began almost immediately, the potatoes had large root systems and broad leaves due to the warmer climate at planting. *Front* and *Heath,* which also showed early invasion, had developed root systems and sprouting leaves. *Malt, Otter* and *Heaths* were planted late March and so the cooler climate would have slowed development.

Levels of Vydate (oxamyl) present (ppm)

Days after application	Site									
	Bigwood	Stockton	Heaths	Round	Common	Searchlight	Front	Crab	Malt	Otter
0	0.88	2.17	3.51	1.14	0.80	1.03	0.75	*	*	1.02
7	0.59	1.03	1.97	0.67	0.37	0.58	0.57	**2.15**	0.89	1.93
14	0.46	0.62	1.24	0.51	0.45	0.35	0.50	1.27	0.70	0.57
21	0.16	**0.93**	0.55	**0.03**	0.48	0.59	**0.40**	1.18	**0.54**	**0.55**
28	0.00	0.49	1.17	0.00	0.44	0.19	0.32	0.46	0.26	0.70
35	0.19	0.46	0.38	0.00	0.31	0.26	0.50	0.39	0.15	0.75
42	**0.20**	0.27	**0.32**	0.00	**0.09**	**0.14**	0.22	0.56	0.00	0.33
49	0.27	0.00	0.72	*	0.11	0.16	0.24	0.07	0.06	0.53
56	0.14	0.00	1.45	*	0.00	0.00	0.13	0.10	0.12	0.28
63	0.23	0.00	0.44	*	0.00	0.00	0.00	0.00	0.00	0.32
70	0.00	*	0.17	*	0.00	0.00	0.00	0.00	0.00	0.23
77	0.02	*	0.16	*	*	0.00	0.00	0.00	0.00	0.09
84	0.00	*	0.00	*	*	*	*	*	*	0.02
91	0.00	*	0.02	*	*	*	*	*	*	0.05

Table 3.6 *Concentrations of oxamyl in the soil (ppm) with days after application*

The bold type corresponds to the time after application when juvenile nematodes were first detected in the roots.

* no analysis made

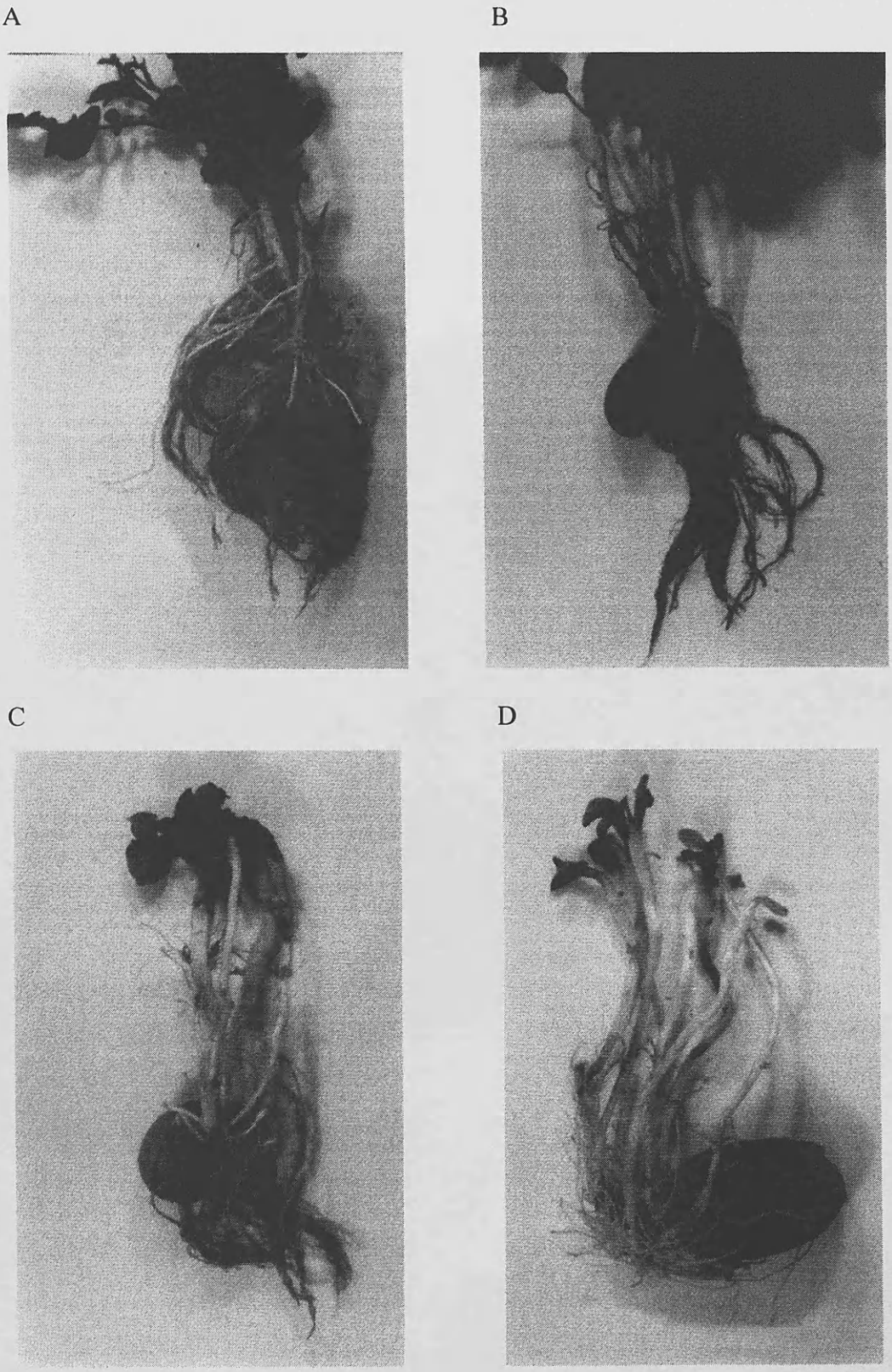


Fig. 3.2 *Development of the potato tuber at each site five weeks after oxamyl application*

Sites = A. Crab, B. Round, C. Stockton, D. Front

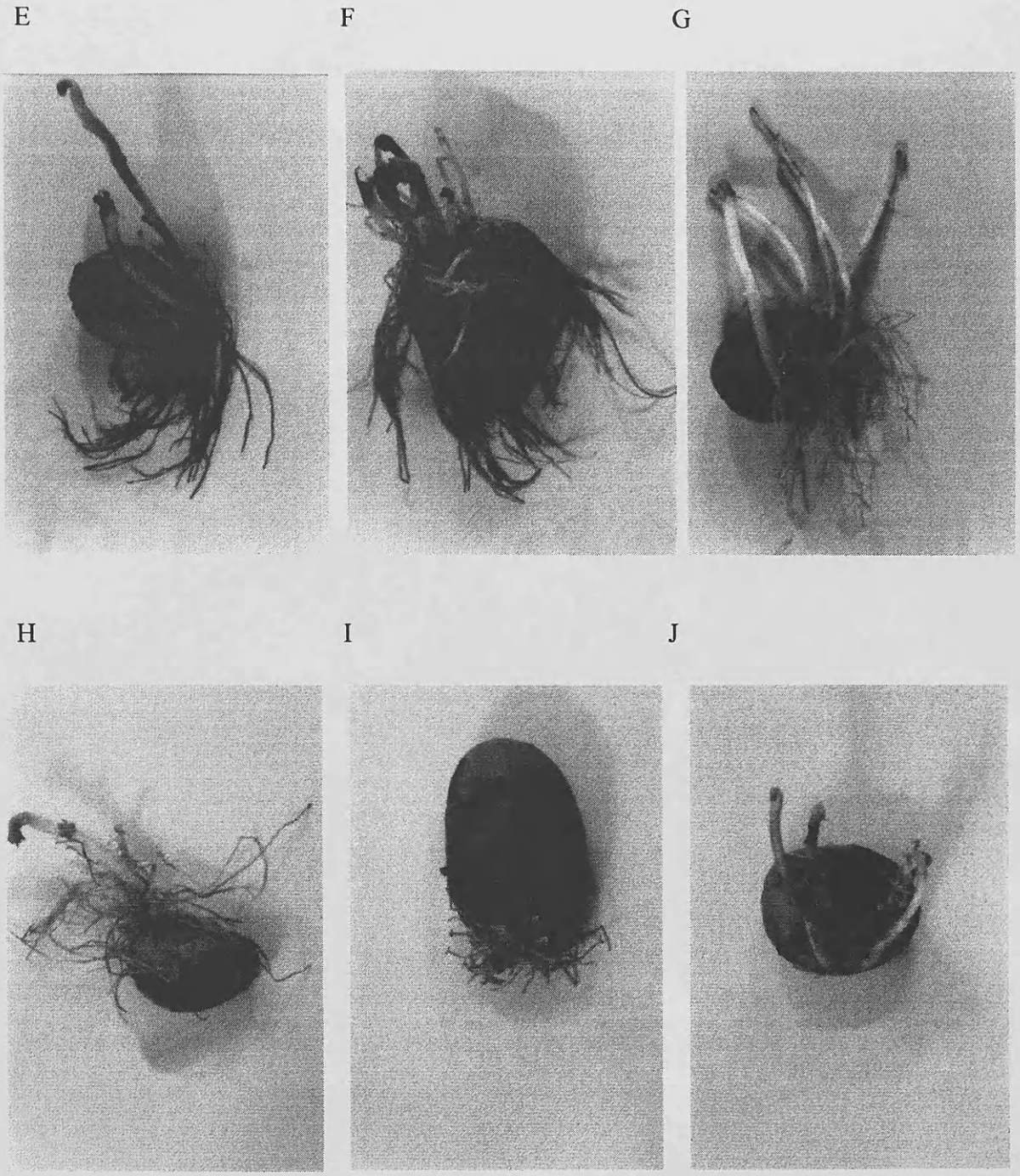


Fig. 3.2 *Development of the potato tuber at each site five weeks after oxamyl application*

Sites = E. Heaths, F. Malt, G. Otter
H. Searchlight, I. Bigwood, J. Common

Little growth at *Searchlight, Common* and *Bigwood* was evident 5 weeks after planting. This was because *Searchlight* and *Common* were planted as unchitted potatoes which would have taken longer to develop. The photograph at *Bigwood* was taken one week after planting but 5 weeks after nematicide application (planting and incorporation were not completed at the same time).

The data in Table 3.2 confirms the rate of tuber development as at the earlier invaded sites, shoot emergence occurred up to 7 days faster than at the later invaded sites. Differences in invasion could simply be related to the development of the potato roots, and the levels of diffusate present. This theory is only based on observational data and so future work would require studies into the impact of root development on nematode invasion and oxamyl control.

At *Crab*, invasion began 7-14 days after application however the oxamyl concentration in the soil was between 2.15 – 1.27 $\mu g\ g^{-1}$. *In vitro* studies showed that 4 hours exposure of *G. rostochiensis* juveniles to 2.0 $\mu g\ ml^{-1}$ oxamyl directly affected motility, and 1.0 $\mu g\ ml^{-1}$ oxamyl caused spasmodic movements, (Evans and Wright, 1982). If these toxic concentrations were applicable in the field a suppression of invasion would have been expected at *Crab*. A possible explanation for this discrepancy could be related to the moisture content of the soil. As discussed in Chapter 2 only 4.5 mm of rainfall occurred within the first 7 days of nematicide application and the soil was not irrigated until July. Whitehead (1987) found that when moisture was readily available to the plant in the top soil, damage to the deeper roots was much less important. However if there was a low water table and the crop was not irrigated in periods of drought, damage to the deeper roots could be important. It is possible that although concentrations of oxamyl were high at

Crab, the lack of moisture prevented leaching through the profile to the deeper roots and so no control was achieved. Invasion continued to week 13 at all sites except *Crab* where further invasion was not observed after 42 days. This could have been caused by rainfall 28 days after the nematicide application, which could have leached the concentrated oxamyl from the surface.

At *Stockton* and *Front* juvenile invasion began early, however development into later stages was not observed for several weeks, peaking at 70 days after planting. This peak in development coincided with large numbers of J3 and J4 stages being present at Searchlight and *Common*. It is possible that development at these four sites was controlled by the rate of heat accumulation in day degrees above a basal temperature. They were all planted within the same week and so the rate of heat accumulation after 70 days only ranged between 520 and 540 day degrees °C (see Table 2.7). These results were similar to Evans (1968), who found that after about 556 °C day degrees had accumulated, most *H. rostochiensis* juveniles in potato roots had developed into young adults. Regression analysis also showed that cumulative temperature had a significant relationship to development (< 0.001) at these sites, however the percentage variation accounted for was wide ($R^2 = 0.76 - 0.21$). *Crab* and *Round* were not significantly related to cumulative temperature, which could be a result of environmental factors such as a lack of leaching at *Crab* and a rapid breakdown of the chemical at *Round* which facilitated rapid and early invasion of PCN at these two sites.

Population increases

Generally at most sites a density dependent relationship was observed with a high Pi (38 – 55 eggs g $^{-1}$) giving a Pf/Pi ratio of 1.3 - 2.1, compared with a low Pi (2 – 13 eggs g ^{1}) where the ratio was 3.3-7.4. (Table 1). Two exceptions were *Otter* and *Stockton* where a decline in the Pf was observed. This suggested that control of PCN had been achieved. At Otter the resistant cultivar Maris piper was grown on a pure *G. rostochiensis* site. This resistance would have hindered pathogen invasion, development and multiplication through a failure of the roots to produce adequate feeding sites for the nematodes (Evans and Haydock, 1990). The invasion data confirms this as numbers invading did not correspond to the high Pi (48 eggs g^{-1}) and development to later stages was not observed. Reasons for the control at Stockton are more difficult to explain. Premiere, a cultivar resistant to *G. rostochiensis* was also grown, however the field population was predominantly *G. pallida*. Invasion levels were high and all stages of development including J5f were observed, the result could be due to sampling error.

At Bigwood and Front the resistant cultivar Maris piper was grown with a mixed PCN population. The increase in the Pf densities suggested that *G. pallida* had successfully developed on the cultivar, confirming its selective reproduction and potential dominance of sites. The increase in Pf populations of 4.5 – 6.6 fold was not reflected in the invasion data as few stages above J3 were observed. This was probably due to the very low numbers of nematodes in the ground at planting.

3.5 CONCLUSIONS

- No significant relationship between invasion and oxamyl concentrations in the soil was found.

- Differences observed in nematode control were associated with the moisture saturation of the soils and subsequent movement and dissipation of the chemical.

- Invasion appeared to be influenced by the rate of development of the potato plant, with more rapid root growth resulting in more rapid invasion.

- The minimum concentration of chemical necessary in the soil to prevent invasion could not be predicted due to fluctuations in oxamyl concentration between sites and within sites.

- Where a *G. rostochiensis* resistant cultivar was grown on sites with pure *G. rostiochiensis*, populations had decreased by the end of the growing season. However, in mixed populations with a resistant cultivar the final populations had increased, demonstrating the ability of *G. pallida* to become dominant in mixed populations if not properly managed.

- Results suggested that development of nematodes was influenced by cumulative temperature as at some sites while invasion began early, development to the next stage was not observed until 70 days after planting following a period of low soil temperatures.

Chapter 4

The validity of comparing nematicide biodegradation in the laboratory to actual degradation rates in the field and the potential for accelerated rates of degradation after repeated applications of nematicide to the same soil

4.1 INTRODUCTION

The majority of degradation studies have investigated the degradation of chemicals in the laboratory, using incubated soils maintained at constant temperature and moisture levels (Smelt and Leistra, 1992). In laboratory experiments the type of chemicals and soils used can be chosen to match that in the field, however the effects of constantly changing weather patterns cannot be paralleled in the controlled environment (Nicholls, 1989). As a result laboratory studies can only be used as an indication of the 'potential' degradation that could occur.

Studies have shown that the development of enhanced degradation may be influenced by the frequency of treatment. Hendry and Richardson (1988) found that soils treated three weeks after a pretreatment hydrolysed 7% of the applied dose of carbofuran in three days. However, after a second treatment (four weeks later), 63% of the dose was hydrolysed after 3 days and after a third treatment (278 h after the second) 92% of the carbofuran dose was hydrolysed within 19 hours. Charnay and Fournier (1994) also found that an initial treatment of carbofuran produced a half-life of 17 days, but after a second treatment the half-life had declined to 8 days

Objectives

- To establish the validity of using laboratory studies to predict actual nematicide biodegradation rates in the field.

- To examine the effects of the frequency of treatments on degradation rates.

4.2 MATERIALS AND METHODS

4.2.1 Soil samples

Soils were taken from the ten farms selected for the field trial (Chapter 2). Samples were taken at three different times of the year:

1. Before oxamyl was applied to the soil (February)
2. Thirteen weeks after oxamyl application (June – August)
3. Approximately 6 months after application (September – October)

The soils taken before planting and after harvest were obtained by taking fifty cores to a depth of 300 mm in a systematic grid pattern, as described in Chapter 3, section 3.2.1. The cores were bulked, mixed and a proportion placed in polypropylene bags to be frozen at -14 °C. The other soil samples were collected 13 weeks after planting by taking 8 soil cores from the 4 stations, bulking them in polypropylene bags and freezing at -14°C.

4.2.2 Determination of available water capacity

The available water capacity (AWC) is the amount of water available in the soil between the two limits of field capacity and permanent wilting point (Davies *et al.* 1993). The soils were held at 70 % of their AWC to enable reliable comparisons to be made. This value was chosen as Suett (1986), and Suett and Jukes (1988) had used ranges of 70-75 % in previous incubation studies.

To determine the AWC soils were defrosted on metal trays at room temperature over night. They were then sieved through a 2mm mesh and 250g samples transferred to weighed and labelled, porous sampling bags. To provide a saturated environment 3 containers (1000

mm high x 500 mm wide) were used. Three sponges were soaked in water and placed at the base of each container. Excess water was then added to a level half way up the sponges. The sampling bags were placed on the sponges and a lid over the containers to prevent evaporation occurring. The soils were then left at room temperature for 48 hours. Following this the sampling bags were removed and weighed. They were then dried by placing in an oven at 105 °C for 24 hours and re-weighed. The AWC was calculated from the gravimetric water content of the soil and the dry bulk density, calculations are shown in Appendix 4.1.

Dry bulk density

The dry bulk density is defined as the apparent density of field soil and is calculated from the oven-dry mass divided by the volume occupied in the field (Hall *et al.*, 1977). To obtain the dry bulk density soils were defrosted as before but NOT sieved. Two hundred and fifty grams of soil were weighed into cylindrical containers, the height of the soil measured and from this the volume calculated as πr^2 x depth. The soils were then oven-dried at 105 °C for 24 hours and re-weighed.

The available water content is often expressed as the volume of water retained between 0.05 bar suction and 15 bar suction as a percentage of the sample volume (Hall *et al.*1977). Therefore to ensure the technique used in this experiment was reliable, preliminary tests were carried out and the results of these are shown in Appendix 4.2.

4.2.3 Incubation Studies

A preliminary study

Before the incubation studies were carried out a preliminary study was made to ensure that freezing soil samples prevented degradation. Soils were obtained from an area outside the laboratory where oxamyl had never been applied. Six hundred grams of air-dried was weighed into a container. To this 2.5 $\mu g\ g^{-1}$ of oxamyl was added as 1.5 ml of 1000 $\mu g\ g^{-1}$ oxamyl solution. The container was shaken and left for half an hour to equilibrate. Fifty grams sub-samples were then weighed into polypropylene bags. Six of the samples were immediately frozen at -14°C. The oxamyl in the other samples was immediately extracted and analysed using methods described in section 2.2.4. The frozen samples were defrosted two weeks later and analysed in a similar manner.

Incubation studies

Accounting for the moisture contents of the soil, proportions equivalent to 800g of dry soil were weighed into containers. To each soil 2.62 $\mu g\ g^{-1}$ oxamyl was added (see Appendix 4.3), in a fume cupboard, as 2.1 ml of a 1000 $\mu g\ g^{-1}$ solution. The containers were shaken gently, then left for half an hour. After this each container was placed on a balance and distilled water accurately added to make the soils up to their calculated 70% AWC. The soils were left for a further half an hour to equilibrate and then mixed thoroughly by placing a screw tight lid on the containers and shaking vigorously for 30 seconds. Soil (250g) was then weighed into three separate containers providing three replicate samples per soil. This resulted in 90 samples in total as from each site there were 3 x replicates of soils before oxamyl application, 3 x 13 weeks and 3 x 6 months. Any excess soil was stored.

Samples were taken immediately by weighing 40 g of soil into labelled bags and freezing. The containers were then re-weighed before perforated foil lids were added and secured with elastic bands. The containers were then placed in the dark in incubators set at 15 ° C. Due to evaporation water was added to the soils twice a week to maintain the 70 % AWC. Samples were then taken 1, 2, 4, 6 and 8 weeks after oxamyl was added. On the day of sampling sub-samples (40g) were weighed into bags and immediately frozen. The containers were then weighed so that the correct moisture content of the soil was known.

4.2.4 HPLC analyses

Residues were extracted and analysed using the methodology outlined in Chapter 2, section 2.2.4.

4.2.5 Analysis of results

A mean result was obtained from the three replicates and from this the percentage of oxamyl remaining was calculated. As regulated conditions were used in the laboratory, degradation followed first order rate kinetics (Bromilow *et al.*, 1980). A Maximum Likelihood Programme (MLP) developed by Lawes Agricultural Trust (1997) for Rothamsted Experimental Station was used to calculate the rate constants and half-lives of the soils. All data were analysed using analysis of variance or regression analysis procedures using Genstat Version 5.1.

4.3 Results

4.3.1 Preliminary studies

The results in Table 4.1 display a significant difference between oxamyl degradation in frozen and unfrozen soils, with the frozen soils containing lower concentrations of oxamyl.

Source	*df*	*MS*	*F*	*F crit*	*P-value*
Soils	2	0.707	3.669	3.285	0.036
Error	33	0.193			
Total	35				

Table 4.1 *ANOVA showing the effect of freezing soil samples on oxamyl degradation*

4.3.2 Comparison of degradation rates

Table 4.2 shows the percentage of oxamyl remaining in the laboratory and in the field with days after application. The breakdown of oxamyl in pre-application soils was used as the laboratory data. Comparison of results showed large differences in the rates of decline. At *Round* after 28 days oxamyl concentrations had only degraded by about 40 % in the laboratory however in the field the oxamyl had completely disappeared. At *Stockton* oxamyl had a half-life of 42 days, but a Dt_{50} of 0-7 days and had disappeared within 56 days in the field.

Data from the laboratory and field were compared using exponential (sympotic regression) or simple linear regression with groups, depending on which analysis gave the highest R^2 values (Figures 4.1 *a-j*). At all sites analyses accounted for over 80 % of the variation (See

Days after application	Environment	Site									
		Bigwood	Stockton	Heaths	Round	Common	Searchlight	Front	Crab	Malt	Otter
0	lab	100	100	100	100	100	100	100	100	100	100
	field	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***
7	lab	83.99	68.50	70.78	83.08	71.61	51.79	58.94	67.99	92.95	76.72
	field	***67.05***	***47.47***	***56.01***	***58.42***	***45.88***	***56.31***	***76.00***	***95.69***	***45.88***	***52.85***
14	lab	67.04	65.19	61.21	76.28	57.20	49.78	57.54	52.05	80.44	62.64
	field	***51.93***	***28.57***	***35.38***	***45.09***	***56.25***	***33.98***	***66.67***	***56.27***	***56.25***	***29.33***
28	lab	53.84	52.99	40.12	61.79	45.76	25.99	49.10	23.42	69.38	56.82
	field	***0.17***	***22.72***	***33.45***	***0.00***	***55.38***	***18.74***	***42.67***	***20.44***	***55.38***	***36.06***
42	lab	35.97	50.27	50.45	52.29	30.51	16.46	27.88	14.92	48.33	44.93
	field	***22.73***	***12.30***	***9.12***	***0.00***	***10.75***	***13.88***	***29.33***	***24.71***	***10.75***	***17.31***
56	lab	21.08	37.93	42.75	23.43	17.80	8.47	19.62	4.50	37.15	42.13
	field	***15.91***	***0.00***	***16.53***	***0.00***	***0.00***	***0.00***	***16.93***	***4.53***	***0.00***	***14.72***

Table 4.2. *The percentage of oxamyl remaining in laboratory and field studies with days after application*

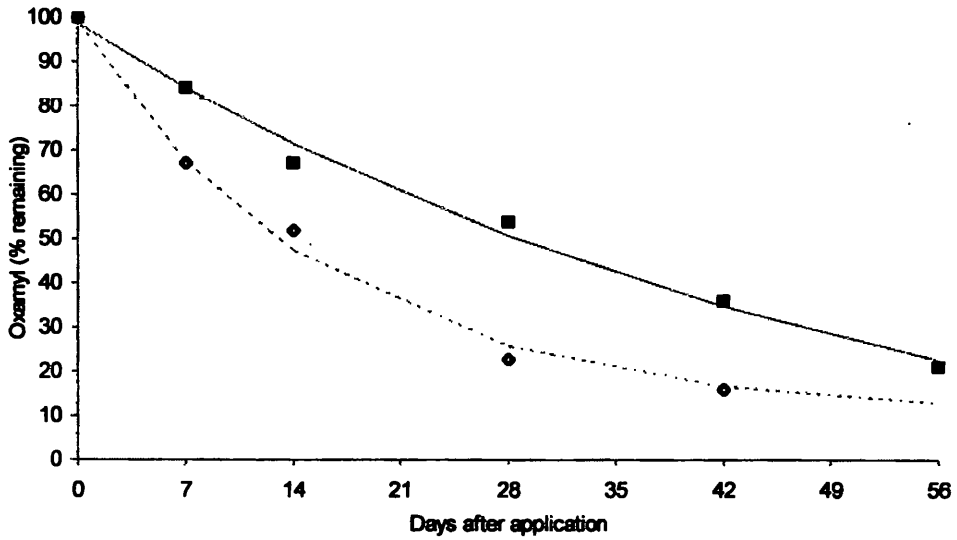


Figure *a* Bigwood
laboratory data (■) = ($y = -17.4 + 115.9 * 0.9812^x$)
field data (◊) = ($y = 13.8 + 86.79 * 0.9337^x$)
Laboratory data significantly different from field data ($P<0.05$)

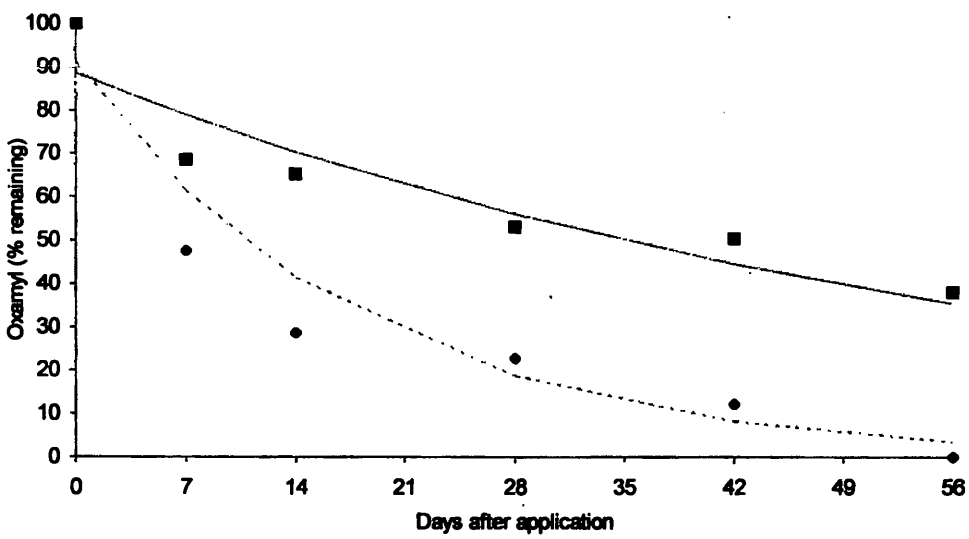


Figure *b* Stockton
laboratory data (■) = ($y = 13 + 88.51 * 0.98379^x$)
field data (◊) = ($y = -0.48 + 91.57 * 0.94565^x$)
Laboratory data significantly different from field data ($P<0.05$)

Figure 4.*1a-j* A comparison of the mean percentage of oxamyl remaining in the laboratory with in the field

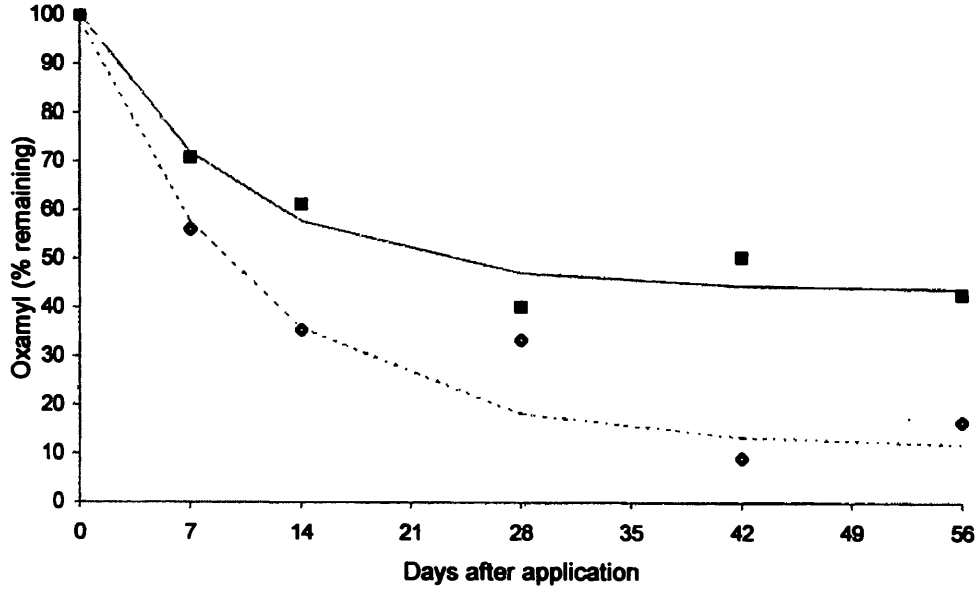


Figure *c* Heaths
laboratory data (■) = ($y = 43.77 + 56.3 * 0.9051^x$)
field data (◊) = ($y = 26.7 + 73.6 * 0.8707^x$)
Laboratory data significantly different from field data ($P<0.05$)

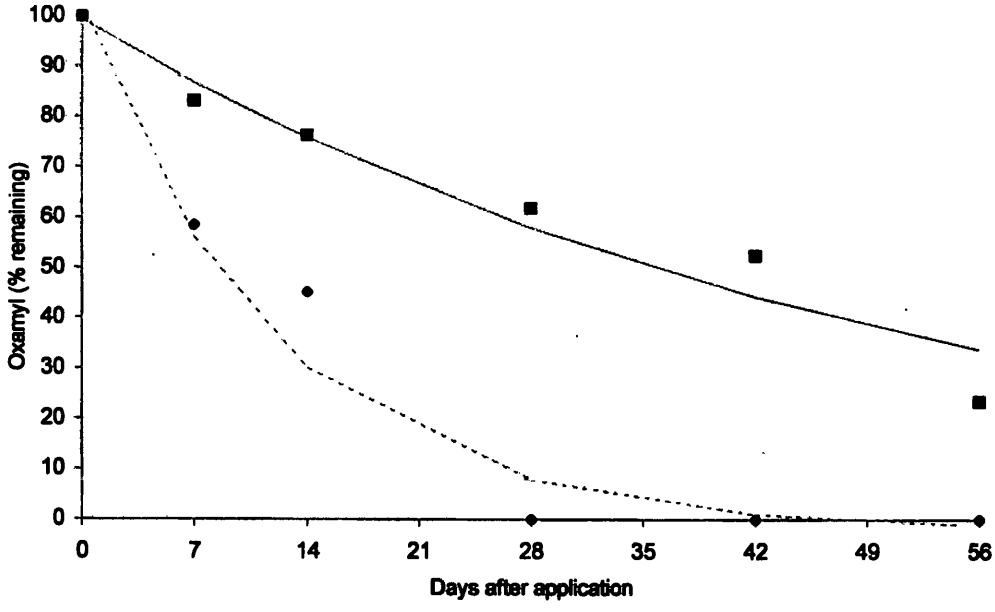


Figure *d* Round
laboratory data (■) = ($y = 43.52 + 54.03 * 0.9267^x$)
field data (◊) = ($y = -2.07 + 104.66 * 0.91923^x$)
Laboratory data significantly different from field data ($P<0.05$)

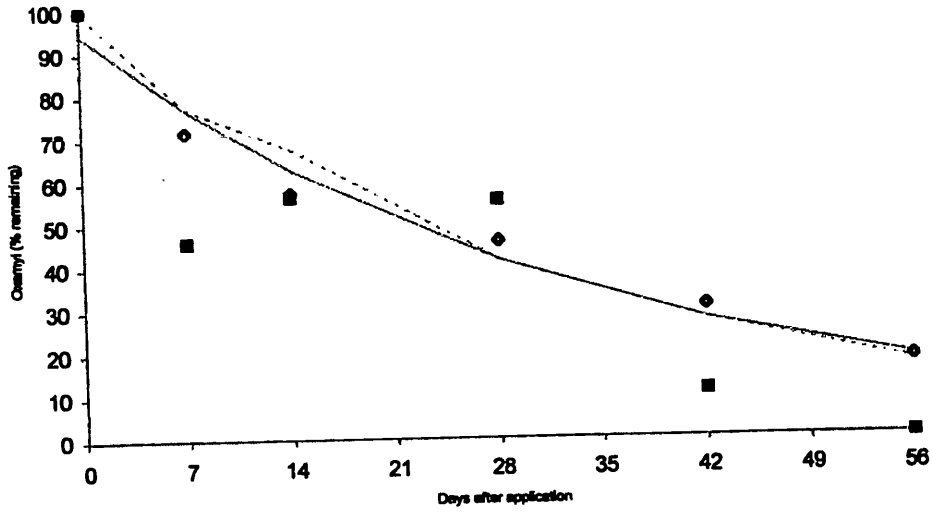


Figure e Common
laboratory data (■) ($y = -8.6 + 107.3 * 0.97^x$)
field data (◊) = ($y = -20.3 + 208.4 * 0.98^x$)
Laboratory data is not significantly different from field data (P>0.05)

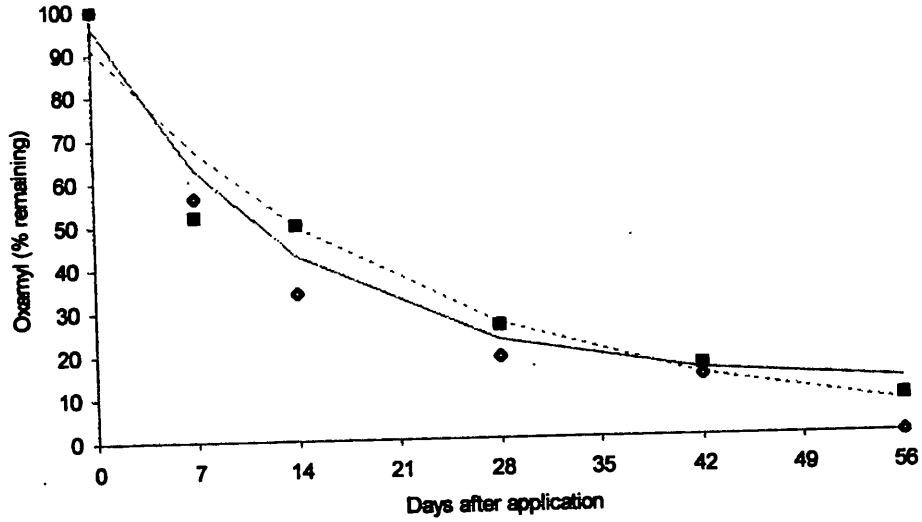


Figure f Searchlight
laboratory data (■) = ($y = 10.91 + 85.35 * 0.9318^x$)
field data (◊) = ($y = 13.98 + 86.16 * 0.902^x$)
Laboratory data is not significantly different from field data (P>0.05)

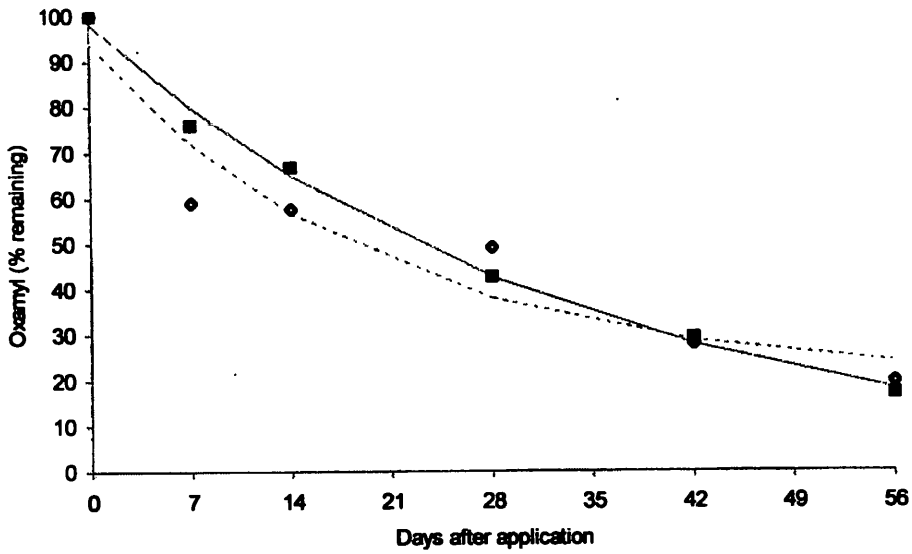


Figure *g* Front
laboratory data (■) = ($y = -1.6 + 99.8 * 0.9714^x$)
field data (◊) = ($y = 74.1 + 19.7 * 0.9513^x$)
Laboratory data is not significantly different from field data (P>0.05)

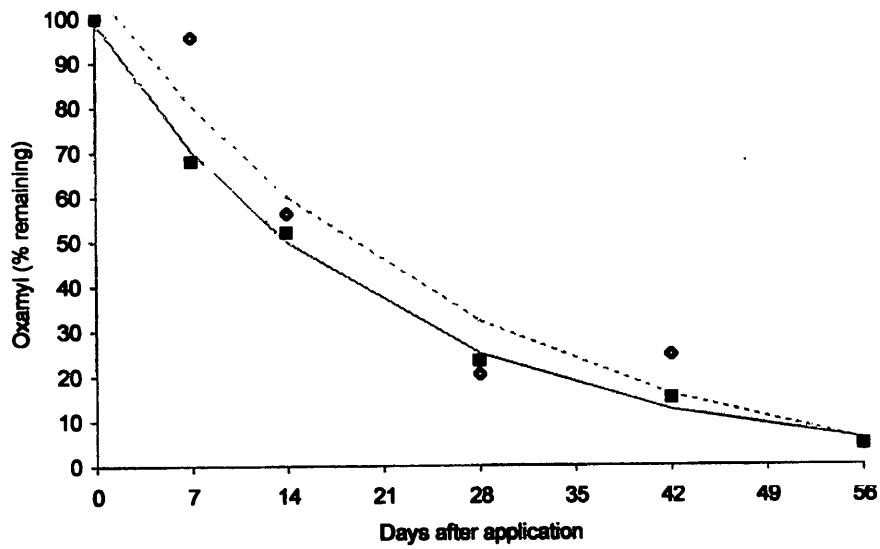


Figure *h* Crab
laboratory data (■) = ($y = -0.6 + 99.8 * 0.9526^x$)
field data (◊) = ($y = -8.7 + 115.5 * 0.9637^x$)
Laboratory data is not significantly different from field data (P>0.05)

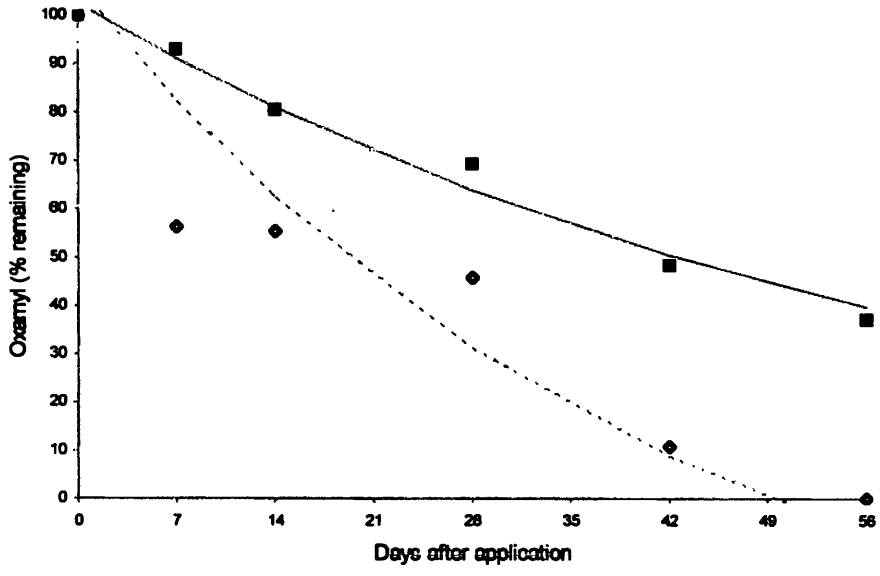


Figure *i* Malt
laboratory data (■) = ($y = -22.7 + 102.56 * 0.98324^x$)
field data (◊) = ($y = -46.9 + 153.5 * 0.9762^x$)
Laboratory data significantly different from field data ($P<0.05$)

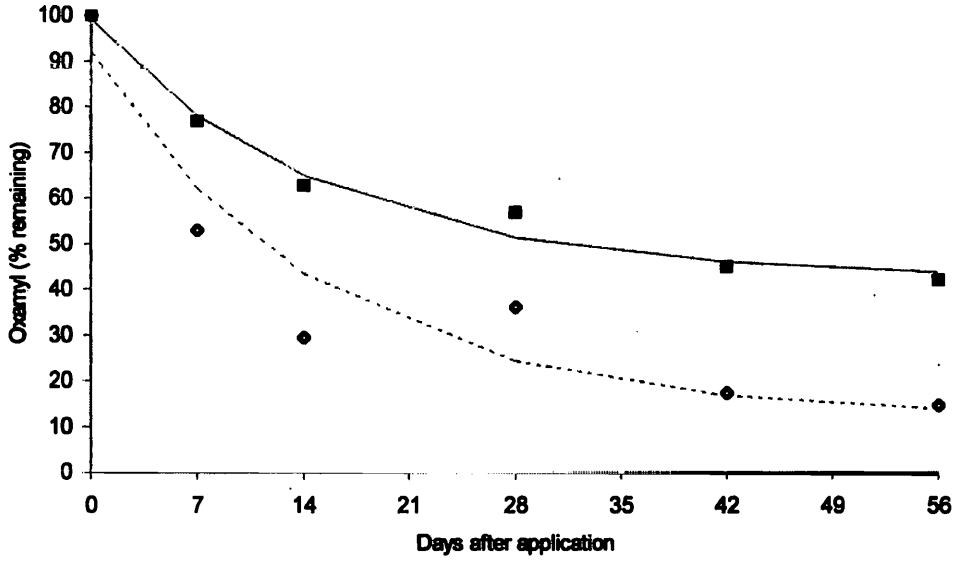


Figure *j* Otter
laboratory data (■) = ($y = 42.44 + 56.53 * 0.9363^x$)
field data (◊) = ($y = 20.6 + 79.13 * 0.8788^x$)
Laboratory data significantly different from field data ($P<0.05$)

Appendix 4.4). Simulations showed that the modelled and field data were not significantly different at Common, Searchlight, Front and Crabstitch. At all other sites degradation in the laboratory was significantly slower to that in the field ($P< 0.05$).

4.3.3 Further treatment of soils with oxamyl

The calculated rate constants and half-lives using the Maximum Likelihood Programme are shown in Table 4.3. It was found that sites could be categorised according to the half-lives of the soil samples. With category 1 sites, the degradative capacity of the soils *increased* in rate from pre-application to 13 weeks, followed by a recovery of soils to more normal breakdown rates at 6 months. Category 2 soils showed an *increase* in degradation rates throughout the season with no recovery whereas with category 3 soils the rate of breakdown declined in week 13 soils followed by an increase at 6 months.

A comparison of the rate constants, with 95 % confidence limits showed a significant difference between soils at the sites in categories 1 and 2 ($P < 0.05$), (Figure 4.2). In category 3 sites the half-lives were not significantly different ($P > 0.05$), apart from at *Malt*, soil B which had an unusually extended half-life of 136 days.

Site	time after oxamyl application	k (day^{-1})	standard error (±)	half-life days	category
Bigwood	Pre-application	0.0250	0.002	27.7	3
	13 weeks	0.0181	0.010	38.3	
	6 months	0.0399	0.008	17.4	
Stockton	Pre-application	0.0163	0.004	42.4	1
	13 weeks	0.1317	0.016	5.3	
	6 months	0.0521	0.013	13.3	
Heaths	Pre-application	0.0171	0.005	40.5	1
	13 weeks	0.2621	0.012	2.6	
	6 months	0.1076	0.023	6.4	
Round	Pre-application	0.0193	0.003	35.9	1
	13 weeks	1.1210	<0.001	0.6	
	6 months	0.1219	0.017	5.7	
Common	Pre-application	0.0293	0.003	23.6	1
	13 weeks	0.3078	0.008	2.3	
	6 months	0.1720	0.017	4.0	
Searchlight	Pre-application	0.0488	0.009	14.2	3
	13 weeks	0.0344	0.004	20.1	
	6 months	0.0443	0.009	15.7	
Front	Pre-application	0.0279	0.006	24.8	2
	13 weeks	0.0773	0.017	9.0	
	6 months	0.1158	0.022	6.0	
Crab	Pre-application	0.0493	0.002	14.1	2
	13 weeks	0.1184	0.035	5.9	
	6 months	0.4053	0.004	1.7	
Malt	Pre-application	0.0169	0.001	41.0	3
	13 weeks	0.0051	0.001	136.0	
	6 months	0.0330	0.004	21.0	
Otter	Pre-application	0.0167	0.003	41.6	3
	13 weeks	0.0134	0.002	51.7	
	6 months	0.0325	0.007	21.3	

Table 4.3 *Rate constants (k) for the degradation of oxamyl in the incubation studies and the degradation categorised according to the half-lives of the three soil samples from each site*

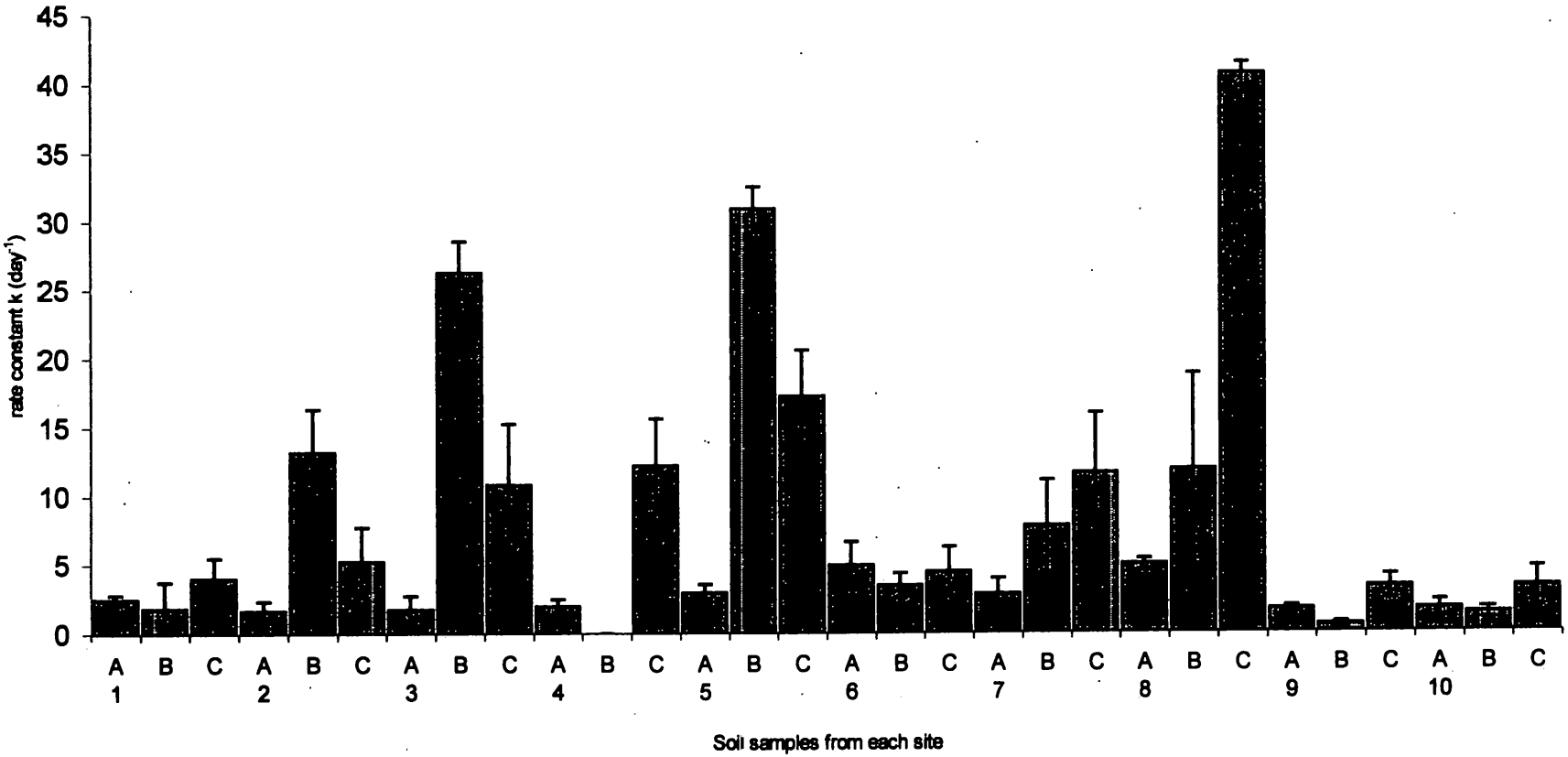


Figure 4.2 *Rate constants (k) for the degradation of oxamyl in incubated soils from 10 sites with three different sampling dates Error bars represent the standard error at a 95% confidence limit*

Sites = 1. Bigwood, 2. Stockton, 3. Heaths, 4. Round, 5. Common, 6. Searchlight, 7. Front, 8. Crab, 9. Malt, 10.Otter
Soils = A. Pre-application soil, B. 13 weeks, C.6 months
Soil Round B was ommitted from the data as the rate constant was much larger than the other data points (= 112.1)

4.4 DISCUSSION

4.4.1 Preliminary studies

Analysis showed that freezing soils did not prevent degradation. However the level of significance (P = 0.04) was quite low suggesting that differences in oxamyl concentrations were minimal between samples. Reasons for this could be either degradation was still occurring but at a slow rate in the frozen soils or some breakdown occurred during the defrosting period. For future studies soils could be defrosted below room temperature to reduce the activity of the microbes.

4.4.2 Laboratory degradation

The degradation of oxamyl in the laboratory was not significantly different to the decline in the field at *Common, Front, Searchlight* and *Crab* (P>0.05). These sites displayed relatively short half-lives ranging from 14 to 25 days, whereas at the other sites longer half-lives of 36 to 42 days were calculated. One exception was *Bigwood* which fell between the two categories (T½ = 28 days), however this site should be considered separately as oxamyl was incorporated several weeks before the potatoes were planted and so the chemical would have been more exposed to factors such as leaching.

Smelt *et al.* (1979) found similar differences in the conversion rates of oxamyl at 15 °C. Half-lives were measured as 13 and 14 days in a clay loam and loamy sand, and 34 and 39 days in a peaty and a humic loamy sand, respectively. They related these differences to the pH of the soil, with a range of 7.1 and 7.4 in the clay loam and loamy sand, and a pH of 5.1 and 5.2 in the humic and peaty sand, respectively. However results from this study are contradictory to the findings of Smelt *et al.*, as *Round* which had the highest pH of 7.0 had

a slow half-life of 36 days, whereas *Crab* with the more acidic pH of 5.9 had a relatively short half-life of 14 days.

Interestingly, the sites where laboratory and field degradation were alike showed a significantly similar decline between the modelled simulations and field degradation (Figures 2.2 a-j). In Chapter 2 it was suggested that this could be due to the texture of the soils. Because they were loamy sands with higher organic matter contents the oxamyl at *Common, Front, Searchlight* and *Crab* may have leached less through the profile compared with the sandier sites. The model only predicted the moisture and temperature in the top 5 cm and did not account for leaching to the deeper profile (Walker, 1987). However *Common, Searchlight, Crab* and *Front* had shorter half-lives in the laboratory which would suggest that some distribution of the chemical had occurred through the profile for degradation to occur (Smelt, 1992).

The shorter half-lives of 14-25 days in some laboratory soils could be related to the viability of microbial degraders. Results from studies using fenamiphos showed degraders still present in the soil three years after the last nematicide application (Stirling *et al.* 1992), and Morel-Chevillet *et al.* (1996) found a degrading capacity to be persisting in a soil five years after carbofuran usage. Results from this study would suggest that microbial adaptation at *Searchlight, Common, Front* and *Crab* had persisted due to the rapid breakdown of oxamyl after a single treatment. However this theory would be difficult to quantify as additional treatments of oxamyl produced very different rates of decline (Table 4.3). Common was a category 1 site where the rate of degradation increased at 13 weeks followed by a recovery to more normal rates at 6 months. *Front* and *Crab* were category 2

soils where rates of degradation increased throughout the growing season, and *Searchlight* was a category 3 soil where no significant change in degradation rates occurred ($P > 0.05$).

Differences in degradation rates could be related to the viability of the microbial degraders. All the soils were stored in sealed bags at 8 – 24 °C (Crop Science dept. max/min). These conditions could have been suitable for less sensitive degraders who were able to remain sufficiently active in the soils, possibly utilising naturally occurring compounds as energy substrates (Hendry and Richardson, 1988). However for others activity under the storage conditions was suppressed. For carbamate pesticides many different degrading stains have been described, with isolates from one soil often differing from those from another soil (Suett *et al.* 1996). Further research would attempt to isolate the microbial degraders in the soils to identify the factors causing the differences in the behaviour of the soils.

4.4.3 Field and Laboratory degradation

Suett *et al.* (1993) claimed that incubation studies only define the *potential* degradative capacity of a soil. However in this laboratory study the degradative capacity of soils was only accurately expressed at four out of the ten sites, and even then results were misleading as the shorter half-lives in the laboratory did not relate to a more rapid decline in the field. The most important factor in nematode research is nematode control. Invasion studies showed that control had particularly failed at *Round* and *Crab; Round* possibly as a result of warm temperatures and substantial moisture, and *Crab* due to a lack of leaching. These were two very different impacts that would not have been detected in the incubation studies. As a result field studies are vital in accurately investigating the behaviour of a chemical.

4.4.4 Further treatments of soils with oxamyl

As shown in Table 4.3, the ability to divide the ten sites into three groups reflects differences in the degradative capacity of microbial populations in the ground. The pattern of category 1 breakdown is similar to the observations of Hendry and Richardson (1988) who found that soils treated 74 days after a first treatment degraded carbofuran more rapidly than those treated 27 and 93 days after a pretreatment. These differences were related to an increase in numbers of carbofuran-degraders between 27 and 74 days, followed by a decrease after 74 days. Although microbial populations were not isolated the soils re-treated 13 weeks after an initial application would appear to be at a higher degradative capacity to the pre-application soils, which had declined by 6 months.

The longer half-lives of the pre-application soils, in categories 1 and 2 would suggest a lag phase where the microbes had to synthesise specific pesticide degrading enzymes to breakdown the chemical (Racke, 1990). The other soil samples from each site had been exposed to oxamyl in the field and so populations would have already been adapted to attain a critical size to degrade the oxamyl at a rapid rate (Kearney and Kellogg, 1985). With category 2 soils the rate of degradation increased throughout the growing season. This could suggest that either the microbes reached their degradative capacity much later, or that populations were able to survive and remain active by utilising alternative naturally occurring compounds (Hendry and Richardson, 1988). Studies of the population abundance of the microbial degraders would have to be made to verify this.

With Category 3 soils no significant difference ($P > 0.05$) was found between degradation rates after further applications. This resistance could be a result of scarcity or poor survival of adapted strains. Charnay and Fournier (1994) found that degradation was twenty times

higher in some soils after a second treatment, however in other soils rates did not increase during the incubation period. Again isolation of the microbes would be needed before conclusions could be reached.

4.5 CONCLUSIONS

- Laboratory results do not always give accurate representations of nematicide degradation rates observed in the field. In this study laboratory and field data was significantly different at six out of the ten sites.

- These sites were the same as the four correctly simulated by the Persist model. This would suggest that differences between modelled and field data were not the result of leaching, as was previous thought, but possibly the result of differences in microbial populations in the soils.

- Monitoring of laboratory soils revealed wide variations in the half-lives and the behaviour of the soils after subsequent additions of chemicals. This demonstrates the complexities of microbial behaviour even under controlled conditions.

Chapter 5

The degradation of oxamyl and aldicarb in soils with an intensive treatment history and the potential for cross adaptation

5.1 INTRODUCTION

The phenomenon of accelerated degradation was first reported 40 years ago for 2,4-dicholrophenoxyacetic acid (Audus, 1949), but its implications were not realised until the early 1970s when important insecticides began to fail in the control of corn rootworm in the USA (Felsot, 1989). In the UK and Europe there has been little indication of accelerated degradation becoming a major threat to cereals or the agrochemical industry. However, during the last decade an increasing number of studies have correlated the reduced efficiency of soil-applied pesticides in the laboratory with accelerated degradation.

In incubation studies at 15 °C, Smelt *et al.* (1987) found that the transformation of oxamyl and aldicarb into non-toxic oximes was enhanced in previously treated, compared with untreated soils. Suett and Jukes (1988) and Suett (1986) also found, with aldicarb and carbofuran respectively, that residues were lost more rapidly from previously treated compared with untreated soils. Autoclaving soils has shown a drastic reduction in the rate of degradation of insecticides, suggesting that accelerated rates could be related to an increased metabolic capacity by soil microorganisms. Studies also imply that pesticides with similar chemical properties could cross adapt, causing similar enhanced transformation rates (Smelt and Leistra, 1992).

Some of the most intensively treated soils in the UK are found in Jersey, where nematicides have been annually applied for at least the past 10 years. If accelerated degradation were a threat to control by granular nematicides, it would be likely to occur at these sites.

Objectives

- To establish whether previous treatments of soil with oxamyl and aldicarb results in the decreased persistence of subsequent nematicide applications.

- To quantify whether enhanced degradation rates are microbial by autoclaving soils.

- To examine the potential for cross-enhancement between the two oximecarbamates, aldicarb and oxamyl

5.2 MATERIALS AND METHODS

5.2.1 Obtaining the soil

Soils were obtained from two sites in Jersey where granular nematicides had been intensively applied, and from corresponding untreated areas close to the treated sites. The soils arrived in sealed polypropylene bags and were stored in a dry room at 8 - 24 °C (Crop Science Dept. max / min).

Year	Soil code: P488 PCN treatment	Soil code: 0222 PCN treatment
1991	Vydate	No treatment
1992	Vydate	No treatment
1993	Vydate	Temik
1994	Vydate	Temik
1995	No record	Temik
1996	Vydate	No treatment
1997	No treatment	Temik

Table 5.1 *Treatment histories of each site*

5.2.2 Soil texture analysis

Methods for particle size analysis (mechanical analysis), organic matter content and pH followed techniques described in Chapter 2.

5.2.3 Determination of available water capacity

The soils were to be held at 70 % AWC, which was determined using a pressure membrane unit and sand suction table as described by MAFF (1982). The soils were sieved through a 4mm aperture and placed into the sand suction table with a Whatmann No. 5 filter paper and left for 48 hours. Water release characteristics under pressure (Pf) were determined at 0.2, 0.3, 0.5, 1.0, 2.1 and 14.64 bar pressures. Methods for dry bulk density and soil moisture content were as described in Chapter 4.

5.2.4 Incubation studies

The incubation studies were set up as follows:

Soil code	Treated / untreated	Autoclaved soil	Chemical to be added
0222	Untreated	No	Aldicarb
0222	Untreated	No	Oxamyl
P488	Untreated	No	Aldicarb
P488	Untreated	No	Oxamyl
0222	Treated	No	Aldicarb
0222	Treated	No	Oxamyl
P488	Treated	No	Aldicarb
P488	Treated	No	Oxamyl
0222	Treated	Yes	Aldicarb
0222	Treated	Yes	Oxamyl
P488	Treated	Yes	Aldicarb
P488	Treated	Yes	Oxamyl

Table 5.2 *Procedures for incubating the soils*

Soils were autoclaved to quantify the impact microbial degradation had on the breakdown of chemicals. Methods for autoclaving are described in the next section.

Accounting for the moisture content, proportions equivalent to 500 grams dry soil were weighed into glass jars. To each jar 10 ml of analytical grade oxamyl or aldicarb was added to give proportions equivalent to 2.6 mg/kg oxamyl and 1.6 mg/kg aldicarb soil dry weight, respectively. These quantities represented the theoretical concentrations when soils are treated at commercial rates. The soils were then mixed by tumbling and allowed to stand for one hour to equilibrate. Distilled water was then added to make the soils up to their calculated 70% AWC. The jars were capped with perforated aluminium foil and stored in the dark at 15°C. Moisture levels were maintained by weekly additions of distilled water. Fifty grams of soil were weighed from each jar 5, 9, 14, 21, 28 and 37 days after treatment. These were immediately frozen at –14 °C.

Autoclaving Soils

Previously treated soils were initially sieved through a 2 mm mesh and the moisture content from a 50 g sub-sample obtained as before (105 °C for 24 hours). Next, six sub-samples of 50 g dry soil were weighed into labelled glass jars. The water content was adjusted to 70 % AWC, accounting for the soil moisture already present. Screw-tight lids were added and the jars shaken for 10 seconds.

The lids were then loosened by a quarter turn and placed in the autoclaver. This was heated to 121 °C, 1.5 bar pressure for 55 minutes. (A thermal indicator was also added to ensure successful sterilisation had been achieved). Once cool, the jars were removed and the lids immediately tightened to prevent contamination. Proportions equivalent to 1.6 mg/kg and 2.6mg/kg soil dry weight of aldicarb and oxamyl were added. This was carried out in a fume cupboard, the jar lids were lifted slightly to add the chemical, and then immediately tightened. The jars were then incubated in the dark at 15 °C. On the

appropriate days after treatment three soils were transferred to labelled bags and immediately frozen at – 14 ° C.

5.2.5 HPLC analysis

The extraction technique for both oxamyl and aldicarb followed that described in Chapter 2.

Oxamyl analysis

A Hewlett Packard Series 1100 High Performance Liquid Chromatography was used for the analyses. The Phenomenox column was a 250 x 46 mm Sphereclone 5u ODS and was held at 30 °C. The mobile phase was 50:50 HPLC grade water and methanol run at a 1.2 ml/min flow rate. A 20 µl injection was monitored at 220nm DAD wavelength with a 3.5 minutes retention time. The analytical efficiency exceeded 95 % and results were not corrected for losses.

Aldicarb analysis

A Merck Lichrocart Lichrosorb column – RP-18 (17 µm) was held at 30 °C. The mobile phase was run as a gradient starting at 10 % acetonitrile and 90 % water, running to 100 % acetonitrile, 0 % water over 8 minutes with a further 3 minute delay between each injection to stabilise the solvents. The solvent was run at a 1.3 ml/min flow rate. A 20 µl injection was monitored at 210nm DAD wavelength with retention times of 2.93 minutes for the sulphoxide, 3.34 minutes for the sulphone and 5.19 for the parent aldicarb. The analytical efficiencies were 111% for the sulphoxide, 101 % for the sulphone and 108 % for the parent aldicarb.

Oxamyl autoclaved soils

Autoclaving soils caused large merging peaks, possibly caused by the breakdown of other soil components at high temperatures. The parent aldicarb was unaffected due to a long retention time and so the same method of analysis could be used, however the oxamyl peak merged with other substances and so a different separation method had to be developed.

The same column for oxamyl was used, held at 30 °C. The mobile phase was run as a gradient starting at 100 % water, 0 % methanol, running to 100 % methanol in 8 minutes with a further 3 minutes delay between each injection. The solvents were run at a 1.2 ml /min flow rate. A 20 µl injection was monitored at 220nm DAD wavelength with a 7.21minute retention time. The analytical efficiency exceeded 95 % and results were not corrected for analytical losses.

5.2.6 Analysing the data

The soils were corrected for moisture content and an average of the three replicate samples calculated. Before the percentage remaining of aldicarb and its degradative components could be calculated the sulphoxide and sulphone had to be adjusted to the molecular weight of the parent aldicarb. The molecular weights were aldicarb (190), sulphoxide (206) and sulphone (222). The rate constants and half-lives of the soils were determined using the Maximum Likelihood Programme as in Chapter 4. The data was analysed using exponential or linear regression analysis procedures using Genstat Version 5.1.

5.3 RESULTS

5.3.1 The degradation of aldicarb

The average concentration of aldicarb, its sulphoxide and sulphone, and the corresponding percentage remaining are shown in Table 5.1 and Figures 5.1 – 5.2. Soils were not analysed immediately after treatment and results are expressed for each sampling occasion as a percentage of the total aldicarb residues 5 days after application. Each result represents the mean of a single analysis of triplicate samples.

Results showed marked and consistent differences in the behaviour of the three toxic residues. With soils from *0222* and *P488*, parent aldicarb was lost rapidly in both the previously treated and untreated soils with 50 % disappearance within 5 days after application. The sulphoxide peaked 9 days after application where it accounted for 83 to 100% of the total aldicarb residue before declining.

Linear regression analysis with groups displayed a significant difference in the rates of breakdown ($P < 0.05$) with the sulphoxide declining faster in *0222T* and *P488T*, compared to the corresponding *0222U* and *P488U* (see Appendix 5.1). The rate of breakdown of the sulphoxide was particularly rapid in the *0222T* soils where an approximate half-life of 7 days was calculated, compared with an approximate half-life of 13 days in the *P488T* soils (Table 5.4a and b).

Soil	DAA	Aldicarb		Sulphoxide		Sulphone	
		ppm	%	ppm	%	Ppm	%
O222	5	0.32	13	2.33	94	0.00	0
untreated	9	0.00	0	2.36	95	0.37	13
	14	0.00	0	2.21	82	0.45	16
	21	0.00	0	1.66	61	0.57	20
	28	0.00	0	1.19	44	0.63	22
	37	0.00	0	0.84	31	0.70	24
O222	5	0.45	18	2.20	82	0.00	0
treated	9	0.15	6	2.34	87	0.00	0
	14	0.00	0	1.56	58	0.00	0
	21	0.00	0	0.72	27	0.00	0
	28	0.00	0	0.14	5	0.00	0
	37	0.00	0	0.00	0	0.00	0
P488	5	0.19	7	2.78	92	0.00	0
untreated	9	0.00	0	3.03	100	0.00	0
	14	0.00	0	1.96	65	0.00	0
	21	0.00	0	1.28	42	0.10	3
	28	0.00	0	0.92	31	0.21	7
	37	0.00	0	0.76	25	0.24	8
P488	5	0.00	0	1.77	76	0.27	11
treated	9	0.00	0	1.78	83	0.60	24
	14	0.00	0	1.24	53	0.74	29
	21	0.00	0	0.73	32	0.73	29
	28	0.00	0	0.14	6	0.53	21
	37	0.00	0	0.00	0	0.41	16

Table 5.3 *Average concentrations of aldicarb, with days after application (DAA), and levels remaining as a percentage of the 5 DAA.*

O222 = soils previously treated with aldicarb and corresponding untreated
P488 = soils previously treated with oxamyl and corresponding untreated

* The average concentrations of the sulphoxide and sulphone were adjusted to the molecular weight of aldicarb before the percentage remaining was calculated.

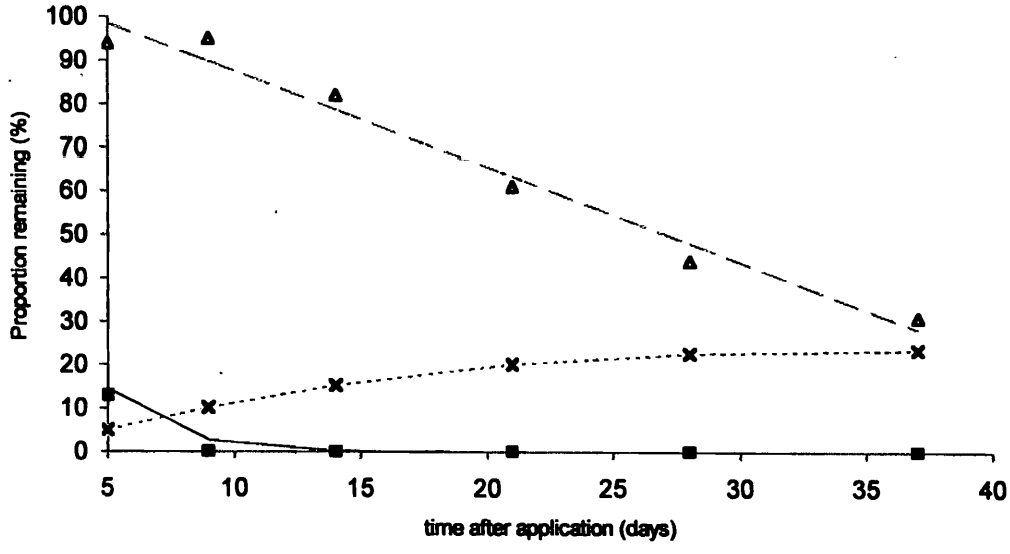


Fig.5.1a *untreated soil (no previous aldicarb treatment)*

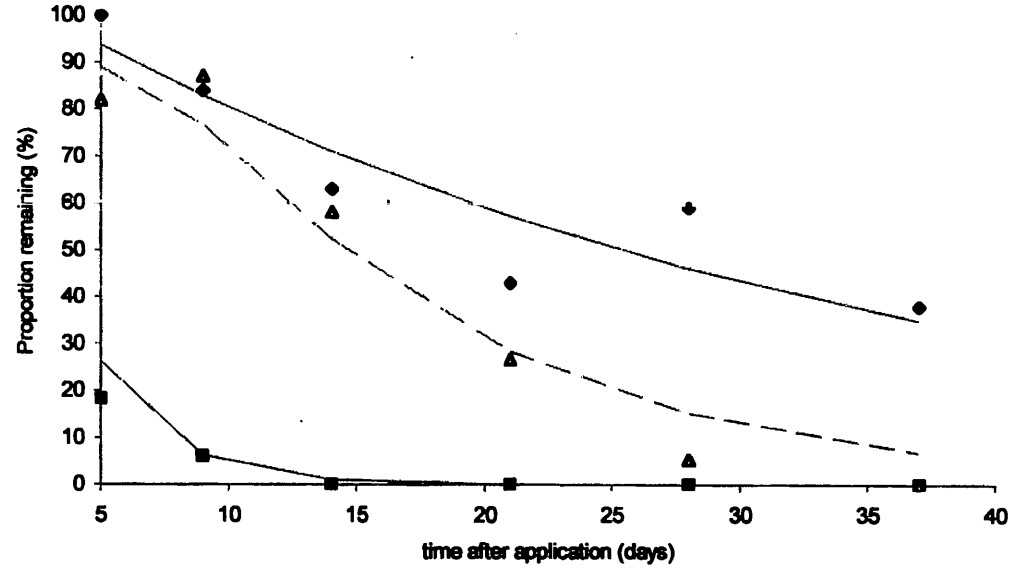


Fig. 5.1b *Soils previously treated with aldicarb - no sulphone was detected*

Fig. 5.1and 5.2 *Disappearance of aldicarb and formation and disappearance of the oxidation products in previously treated and untreated soils*

(□ = parent aldicarb, Δ = sulphoxide, × =sulphone, ◊ =parent aldicarb in autoclaved soil)

Aldicarb sulphoxide in treated and untreated soils were significantly different ($P < 0.05$)
Untreated = $y = (109.48 + -2.192^{*} x)$ Treated = $y = (118.28 + -3.026 x)$

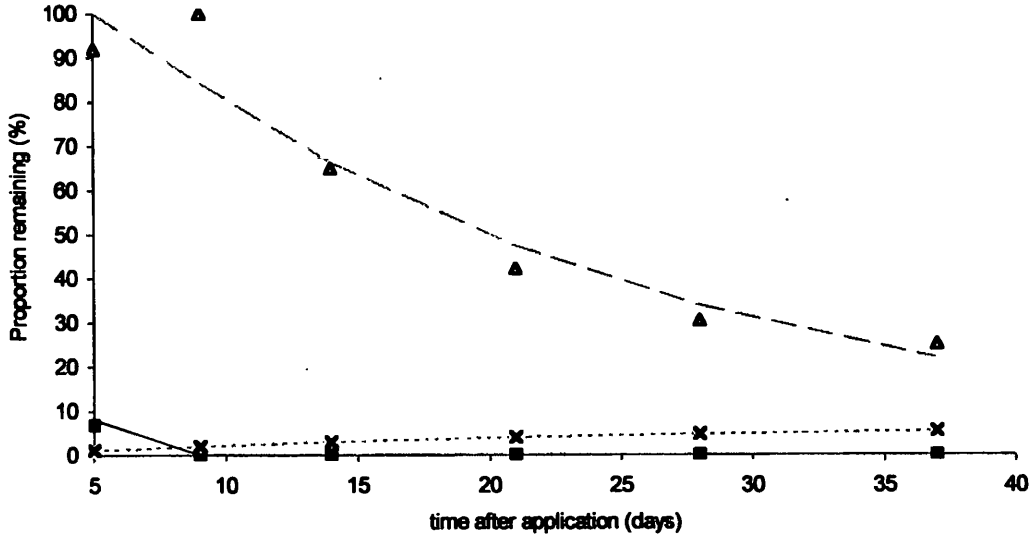


Fig.5.2a. *untreated soil (no previous oxamyl treatment)*

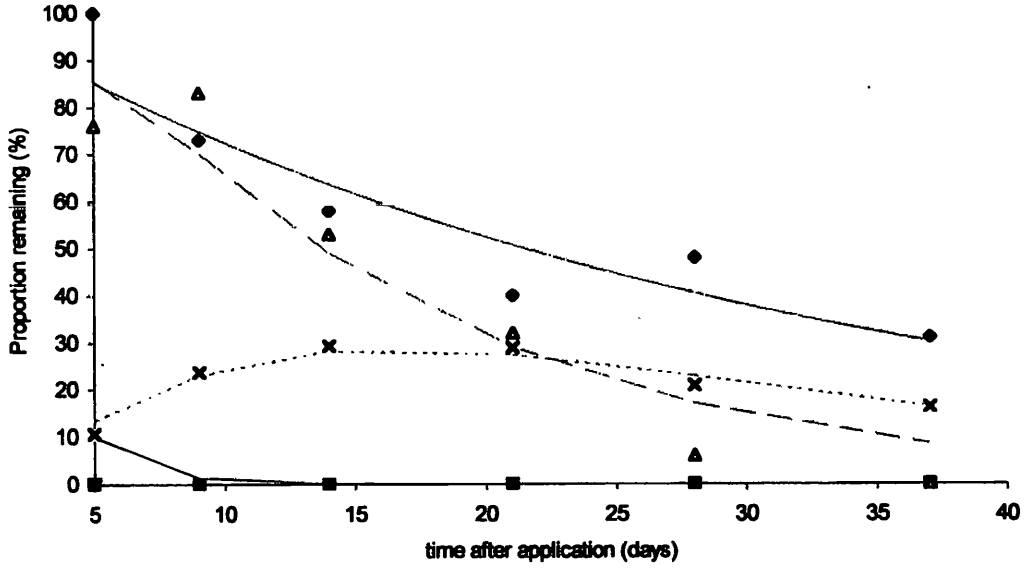


Fig. 5.2b *Soils previously treated with oxamyl*

(□ = parent aldicarb, Δ = sulphoxide, × = sulphone, ◊ = parent aldicarb in autoclaved soil)

Aldicarb sulphoxide in treated and untreated soils were significantly different (P <0.05)

Untreated = (y = 106.02 + -2.466 x)
Treated = (y = 94.82 + -2.796 x)

Chemical application	soil type	Rate coefficients (day-1)				
		k1	*k2*	*k3*	*k4*	*k5*
Aldicarb	0222U	0.43	0.02	*	0.02	0.02
	0222T	0.32	0.00	0.04	0.09	*
	P488U	0.14	0.00	0.79	0.05	*
	P488T	0.50	0.05	*	0.03	0.07

Table 5.4a. *Rate constants (k) for the degradation of aldicarb*

Chemical application	soil type	standard error (±)	half life (days)		
			parent	aldicarb	aldicarb
Aldicarb	0222T	0.08	1.62	34.65	34.65
	0222U	0.08	2.17	7.74	*
	P488T	0.08	4.95	13.86	*
	P488U	0.08	1.39	23.1	9.9

Table 5.4b. *The standard error and half-life of aldicarb and its metabolites (days)*

Chemical application	soil type	k (day-1)	standard error (±)	half-life (days)
Oxamyl	0222T	0.04	0.00	17.30
	0222U	0.04	0.01	17.30
	P488T	0.07	0.01	9.90
	P488U	0.11	0.01	6.30

Table 5.4c. *Rate constants (k) for the degradation of oxamyl in the incubation studies*

Chemical application	soil type	k (day-1)	standard error (±)	half-life (days)
	P488T	0.18	0.05	3.82
Aldicarb	0222T	0.03	0.01	23.10
	P488T	0.04	0.01	17.33

Table 5.4d. *Rate constants (k) for the degradation of oxamyl and aldicarb in autoclaved soils*

O222T = previously treated with aldicarb, 0222U = untreated
P488T = previously treated with aldicarb, P488U = untreated
* Unobtainable results

Sulphone was not detected in the *0222T* soils, however in the *0222U* soil levels comprised 24 % of the total residue after 37 days. In the *P488T* soils sulphone comprised 29 % of the total residue at 14-21 days after application before declining, however with the *P488U* soil the sulphone had only accounted for 8 % of the residue by 37 days. Autoclaving resulted in a much slower breakdown of parent aldicarb with 30 to 40 % still detected 37 days after application.

The decline of the 'total' aldicarb residues is shown in Figures 5.3. Linear regression analysis with groups, or exponential (sympotic regression), depending on the better R^2 value, showed a significant difference between the degradation of total aldicarb residues in *0222T* and *0222U* ($P > 0.05$). However with oxamyl pretreated and untreated soils no significant difference in decline rates was found (Appendix 5.2).

5.3.2 The degradation of oxamyl

Table 5.5 shows the average concentration and percentage remaining of oxamyl in the previously treated and corresponding untreated soils. Results were again expressed for each sampling occasion as a percentage of the total residues 5 days after application. Exponential regression analysis (Figure 5.4 and Appendix 5.3) did not display a significant difference between the breakdown of oxamyl in *P488T* and *P488U* soils ($P < 0.05$) and similarly between *0222T* and *0222U* soils ($P < 0.05$). However the half-life of oxamyl in the *0222T / U* soils was much longer at approximately 17 days compared to a range of 6 – 9 days in the *P488T / U* soils, (Table 5.4c).

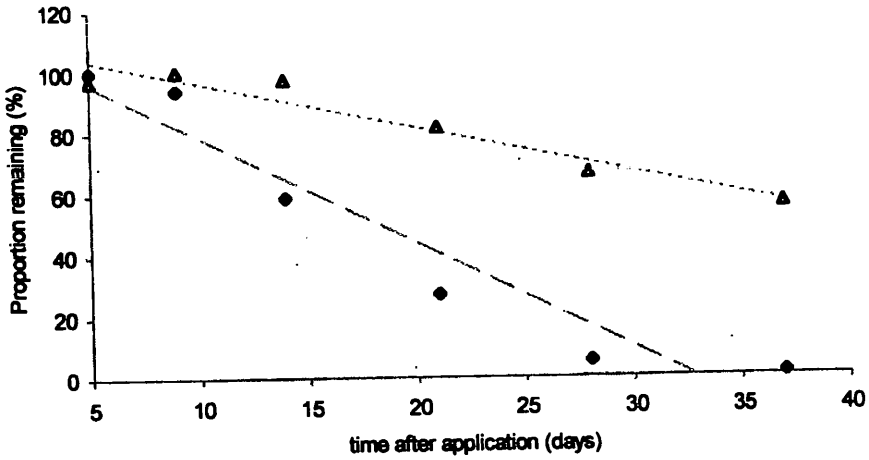


Figure 5.3a *Comparing the decline of total aldicarb in aldicarb pretreated and untreated soils*

Δ = untreated and ◊ = treated soil
Untreated = y = 110.99 + 1.4622x
Treated = y = 113.15 + -3.4439x
Untreated data was significantly different from treated data ($P < 0.05$)

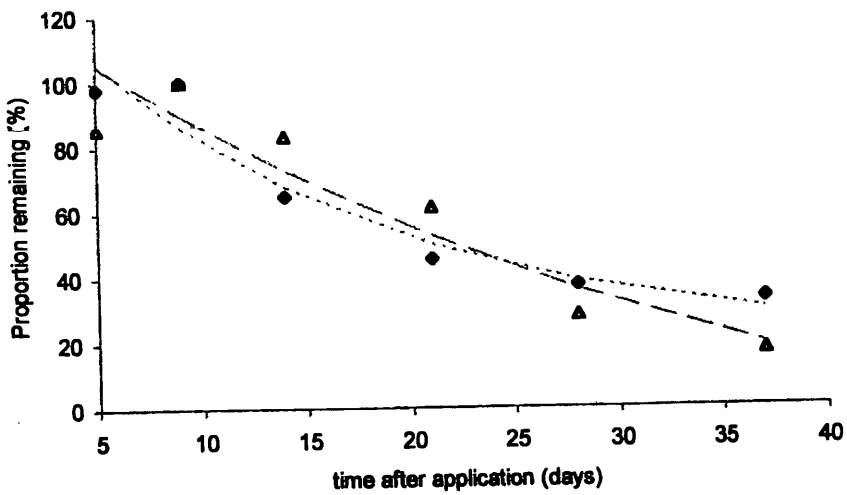


Figure 5.3b *Comparing the decline of total aldicarb in oxamyl pretreated and untreated soils*

Δ = untreated and ◊ = treated soil
Untreated = y = -41.13 +167.8 * 0.9727^{x}
Treated = y = 18.5 + 120 * 0.9384^{x}
Untreated data was not significantly different from treated data ($P > 0.05$)

Soil	DAA	Oxamyl		Autoclaved soil			
				aldicarb		oxamyl	
		ppm	%	ppm	%	ppm	%
O222	5	2.80	100				
untreated	9	2.37	85				
	14	1.73	62				
	21	1.82	65				
	28	0.76	27				
	37	0.74	26				
O222	5	3.64	100	1.78	100	1.76	100
treated	9	3.39	93	1.49	84	1.38	78
	14	2.36	65	1.12	63	1.1	62
	21	1.93	53	0.77	43	1.15	65
	28	1.27	35	1.06	59	0.89	51
	37	1.05	29	0.68	38	0.45	25
P488	5	6.00	100				
untreated	9	4.58	76				
	14	2.39	40				
	21	2.03	34				
	28	1.25	21				
	37	0.86	14				
P488	5	3.28	100	1.86	100	2.22	100
treated	9	1.73	53	1.36	73	0.74	33
	14	1.20	37	1.08	58	0.81	37
	21	0.73	22	0.73	40	0.00	0
	28	0.40	12	0.89	48	0.00	0
	37	0.00	0	0.58	31	0.00	0

Table 5.5 *Average concentrations of oxamyl, with days after application, (DAA) and levels remaining as a percentage of the 5 DAA. Average concentrations of aldicarb and oxamyl remaining in autoclaved soils*

O222 = soil previously treated with aldicarb and corresponding untreated
P488 = soil previously treated with oxamyl and corresponding untreated

Only treated soils were autoclaved

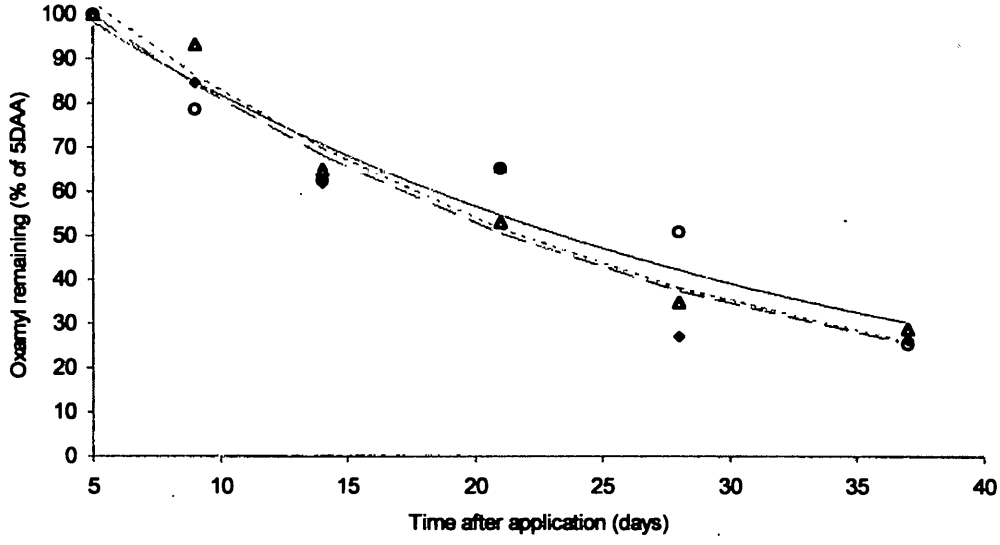


Figure 5.4a Comparing the *decline of oxamyl in aldicarb treated, untreated and autoclaved soils*

Δ = untreated, ◊ = treated soil and o = oxamyl in autoclaved soils
Untreated = $y = -12.5 + 132.8 * 0.9656^x$)
Treated = $y = 6.2 + 123.3 * 0.9529^x$)
Untreated data was not significantly different from treated data (P > 0.05)

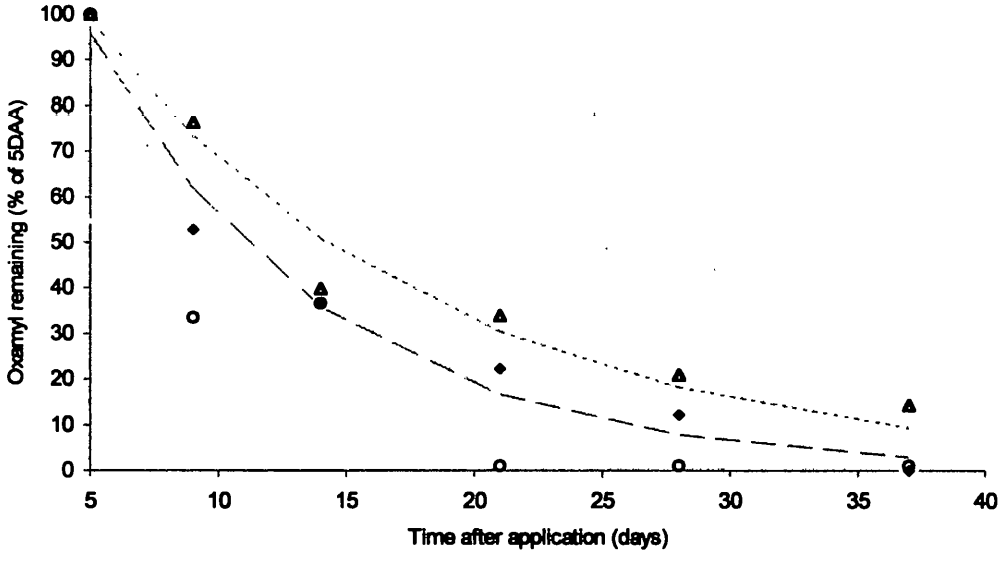


Figure 5.4b *Comparing the decline of oxamyl in oxamyl treated, untreated and autoclaved soils*

Δ = untreated, ◊ = treated soil and o = oxamyl in autoclaved soils
Untreated = $y = 4.27 + 173.1 * 0.8824^x$)
Treated = $y = 12.22 + 149.2 * 0.9019^x$)
Untreated data was not significantly different from treated data (P > 0.05)

Autoclaving of the soils produced some unexpected results (Table 5.5). When oxamyl was added to the autoclaved *0222T* soils the rate of breakdown was similar to the unautoclaved soils with a half-life of about 23 days (Table 5.4c). However when oxamyl was added to the autoclaved *P488T* soils the rate of breakdown was accelerated with a half-life of only 4 days.

Soil texture analysis (Table 5.6) found that *0222T/U* were of a loamy sand texture, whereas *P488T/U* soils had a higher clay content. At both sites organic matter was much higher in the untreated soils, but the pH varied with a lower pH of 4.8 at *0222U* than at 0222T (5.4), compared with a higher pH of 6.1 at *P488U* than at *P488T* (4.9). Autoclaving resulted in an increase in the pH of treated soils at both sites.

Treatment		Soil type	Sand (%)			Clay (< 2 μm) (%)	Silt (2-50 μm) (%)	Organic matter (%)	pH
			coarse	medium	fine				
0222U		sandy loam	7.31	7.79	63.69	14.66	2.43	7.30	4.82
0222T		loamy sand	5.75	9.88	69.33	13.68	1.45	2.04	5.39
0222T	autoclaved	sandy loam/l	5.18	10.07	67.59	10.12	1.23	2.15	6.13
P488U		sandy clay lc	11.19	10.81	58.21	19.80	2.74	5.95	6.05
P488T		sandy clay lc	4.48	5.27	66.41	20.77	4.43	3.13	4.85
P488T	autoclaved	sandy clay lc	5.79	5.34	57.17	27.37	7.36	3.08	5.86

Table 5.6. Soil properties at each site

5.4 DISCUSSION

The rapid loss of parent aldicarb followed by a more stable decline of the sulphoxide corresponds with the findings of Suett and Jukes (1988). The fact that no aldicarb sulphone was detected in some soils is puzzling, however Smelt *et al.* (1987) also failed to detect sulphone in certain soils.

The significantly faster degradation of aldicarb sulphoxide in pretreated soils reflects the findings of Suett and Jukes (1988) who suggested that the rapid breakdown of the treated soils was a result of microbial enhancement. The drastically reduced rate of transformation of parent aldicarb in the autoclaved soils would indicate that that the processes involved, could be assumed to be microbial (Stirling *et al.*, 1992).

The significantly faster breakdown of aldicarb in oxamyl treated (*P488T*) compared with untreated soils would indicate that cross-adaptation occurred. Similar observations were found by Smelt *et al.* (1987) who found that the transformation of aldicarb was distinctly faster in soils previously treated with oxamyl.

Results for the degradation of oxamyl were surprising as the oxamyl degraded at significantly similar rates between treated and untreated soils in both *P488* and *0222* soils suggesting that enhanced degradation was not occurring. Cross adaptation also seemed unlikely to have occurred as the half-life of oxamyl in the *0222* soils was much longer at 17 days compared with 6-9 days in the *P488* soils.

The similar decline of oxamyl in the *0222T* and *0222U* soils could possibly be explained by the higher pH of 6.05 in *0222U* compared to pH 4.85 in the *0222T* soils (Table 5.4).

Harvey and Han (1979) demonstrated that the stability of oxamyl in aqueous solution was considerably less at a higher pH than at 4.7. Smelt *et al.* (1996) also found that aldicarb, oxamyl and ethoprophos were degraded quicker in a sandy soil of pH 7.3 than of pH 5.6. The higher pH in the *0222U* could have compensated for enhanced rates of microbial degradation in the *0222T* soils due to increased levels of hydrolysis (Smelt and Leistra, 1992). Laboratory studies comparing the rates of chemical breakdown in previously treated and untreated soils of differing pH levels would help quantify this theory

The results for the additions of oxamyl to autoclaved soils was surprising, and it could be suggested that soil sterilisation had not been successful. However this is not likely as each set of data was the result of the mean of three replicate samples and the standard errors between these were low ($P < 0.03$). It is possible that the breakdown of oxamyl could be associated more with abiotic transformation processes rather than microbial degradation. Hydrolytic transformations are important abiotic reactions, which occur between many pesticides and reactive inorganic and organic compounds in solution. They can be base or acid catalysed in nature and often occur through interactions with reactive chemical groups exposed on clay mineral surfaces (Racke, 1990).

The main pathway for the degradation of oxamyl is by hydrolysis of the carbamate group and so hydrolytic transformations are possible. The degradation of aldicarb to its sulphoxide and sulphone is primarily oxidation, coupled with some hydrolysis to by-products. (Bromilow *et al.*, 1980). Texture classification showed the oxamyl soils were of a higher clay consistency which is known to be large contributor to degradation by abiotic processes (Racke, 1990). Further chemical studies would be need to be undertaken to verify this theory.

Results demonstrate the variety of chemical behaviour that can occur. In this study application technique, temperature and moisture were all constants however uncontrollable variables such as pH and soil texture still had an impact on the breakdown of the chemicals. Wth soil-applied pesticides the activity of the chemicals can be influenced by a range of biotic and abiotic processes, most of which interact so extensively that the contribution of any single variable is usually difficult to discern (Suett *et al.*, 1996).

5.5 CONCLUSIONS

- Previous treatments of soils with aldicarb showed enhanced rates of breakdown compared to untreated soils, however with oxamyl no significant decline was observed.

- Autoclaving of aldicarb soils resulted in an extended persistence of parent aldicarb strongly suggesting that degradation was microbial, however for oxamyl autoclaving had no effect on the rate of breakdown.

- It was suggested that oxamyl degradation occurred as a result of abiotic transformations , however further studies would be needed with other soils to assess this theory.

- Cross enhancement was found for aldicarb, which declined as rapidly in oxamyl treated compared with aldicarb treated soils. Oxamyl also degraded in aldicarb treated soils but the decline was slower than in oxamyl treated soils.

Chapter 6

Final Discussion

6 FINAL DISCUSSION

Research from this study into 'the degradation of soil applied nematicides used for the control of potato cyst nematodes' displayed the complexities of both the soil and the environment on oxamyl degradation.

Research into the rate of degradation of oxamyl in field soils revealed wide variations in nematicide persistence. The fact that one site was degrading significantly faster than the others suggested that enhanced breakdown could be occurring. Reasons for this degradative capacity were suggested to be a result of a high pH, warm temperatures and moist soils. However in the laboratory, when moisture and temperature were constant a second treatment of oxamyl produced an extremely rapid breakdown of the chemical with a half-life calculated to be 0.6 days. This would imply that microbial factors could also be involved in the rapid breakdown of the chemical. A simple experiment to verify this would be to sterilise the soils by autoclaving using methods described in Chapter 5, then repeating the incubation methods of Chapter 4.

Population density studies showed the rapidly degrading site had a high population of 40-50 eggs g^{-1} and species displayed mixed populations of *G. pallida* and *G. rostochiensis*. Results from Chapter 3 demonstrated the ability of *G. pallida* to become dominant in a mixed population if not properly managed. As a result it could be strongly recommended that if soil sterilisation suggested degradation was microbial, then it would be important to recommend that the grower used a soil sterilant such as 1,3 dichlorpropene prior to the planting of potatoes in the field.

Unfortunately the moisture content and temperature of a field can not be controlled. However results from the studies in Chapter 2 suggested that growers should plant their tubers as early in the season as possible, before temperatures rise, as results would suggest that development of the nematode was controlled by cumulative temperature in day degrees.

Results from Chapter 3 would also imply that the planting of unchitted potatoes should be recommended, as invasion appeared to be influenced by the rate of root growth. To verify this theory studies could be carried out with various chitted and unchitted potato cultivars in pot experiments containing oxamyl treated soils. Weekly root invasion studies and analysis of the oxamyl concentrations could be made to ascertain the impact root development had on invasion.

It was concluded that the laboratory results did not always give an accurate representation of nematicide degradation rates observed in the field. However the fact that four out of ten sites gave an accurate representation would suggest that if adequate numbers of replicates were used, then a 'potential' indication of behaviour in the field could be obtained.

The treatment of soils with additions of oxamyl 13 weeks and 6 months after initial applications displayed interesting differences in the pattern of breakdown of the chemicals. To continue this research further it would be important to identify the microflora in the soils. Studies could use antibacterial and antifungal antibiotics such as chloramphenicol and cyclohexamide, respectively to identify if the components were bacterial or fungal.

If bacterial further studies could include most probable number (MPN) tests as carried out by Hendry and Richardson (1988). Using minimal agar with oxamyl as the sole carbon source the levels of CO2 emitted could be assessed to obtain an MPN count. Dilution plate counts on minimal agar with oxamyl would also indicate the number of pesticide degrading bacteria in the soils (Parekh *et al.*, 1992). Ultimately a diagnostic assay would want to be developed to identify the microbial strains involved in enhanced degradation, and from this a prediction of the potential for accelerated breakdown of chemicals in a particular soil should be possible.

Results from Chapter 5 displayed the potential for cross-enhancement of aldicarb in oxamyl treated soils. This result emphasises the importance of avoiding even trace contamination of compounds with other soils, particularly as Harris *et al.* (1984) found the ability to degrade carbofuran rapidly could be induced in a previously untreated soils by transferring as little as 0.25% of an active soil.

Chapter 7

References, Appendices

REFERENCES

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APPENDICES

Appendix 2.1 Questionnaires sent to the growers

This questionnaire will enable analyses of the soil and root samples collected from your potato field.

NAME: FARM:

1. What variety of potato was grown this year?

2. What was the planting date?

3. What was the potato planting depth?

4. What rate of Vydate was applied to the field?

5. How was the Vydate incorporated?

6. Was a destoner used?

7. Did you irrigate? When and how much?

8. Have you ever used soil sterilants and potato treatments on the field? If yes when and what?

9. What is your potato rotation length?

10. What crops have been grown on the field within the last potato rotation?

11. What pesticides have been used on the field within the last rotation?

12. How many years have potatoes been grown in the field?

13. Has Vydate always been used? If not what other nematicides have been used and when?

Appendix 2.2 Soil Analyses

Dispersing agent – dissolve 50g of sodium hexmetaphosphate and 7g anhydrous sodium carbonate in water and dilute to 1 litre. Determine *R* the weight of residue obtained by pipetting 10 ml into a previously dried and weighed basin and evaporate to dryness on a hotplate. Place the basin in an oven for 4 h at 102 °C. Cool the beaker in a desiccator and weigh *(r = 0.639).*

Calculations

In all calculations the weight of peroxide treated soil was needed. This was the weight of soil after removal of organic matter:

Weight of peroxide soil = weight of coarse sand + weight of medium sand + weight of fine sand + ((20 x weight of silt + clay) minus r)

Calculating the moisture content of the soil

For each calculation the hygroscopic moisture had to be accounted for:

Weight of crucible (a)
Weight of crucible and air-dry soil (b)
Weight of crucible and oven-dry soil (c)

$$\text{Moisture content} = \frac{\text{loss in weight (b-c)}}{\text{Weight of air-dry soil}} \times 100$$

Calculating the Coarse sand (0.6 –2.0 mm fraction)

% coarse sand = (A / weight of peroxide treated soil) x 100

A = weight of coarse sand.

Medium sand fraction (0.6 – 0.212 mm) *Fine sand (0.212 – 0.0693 mm)*

Calculated in a similar manner to coarse sand.

Silt and clay fraction (< 0.063 mm fraction)

% 0.063 mm fraction = (100 (20 B – r)) / weight peroxide treated soil

B = weight of 0.063 mm fraction

Clay fraction

% clay = (100 (20 C – r)) / wt. peroxide treated soil C = weight of clay fraction

Silt % silt = (% silt = clay) - % clay

Organic matter content

Hygroscopic moisture – (loss in weight / weight of air-dried soil) x 100

Loss on ignition = (loss in weight / weight of oven dry soil) x 100

Appendix 2.3 Calculating the accumulated temperature in day degrees

Tmax = Maximum temperature of the day

Tmin = Minimum temperature of the day

Base (B) = Lowest tolerable temperature for each species

= 4 °C for *G. pallida* and 6 °C for *G. rostochiensis* (Haydock, pers.com.)

1. When Tmax and Tmin were both above base (B), the effective daily contribution was

   (Tmax + Tmin) / 2 –B

2. When Tmax and Tmin were both below B, the effective daily contribution = 0.

3. When Tmax was greater than B and Tmin was less than B then:

   (Tmax-B) / 2 – (B-Tmin) / 4

4. If the mean was less than B, the effective daily contribution was (Tmax – B) / 4

Appendix 2.4 HPLC Analyses

Making the serial dilutions for the calibration curve:

Concentration of standard (ppm)	Amount of standard required (ml)	Serial dilution made (ppm)
50	5	5 ppm
50	2.5	2.5 ppm
50	1.5	1.5 ppm
50	1	1 ppm
50	0.5	0.5 ppm

Standard concentration (50 ppm) x amount of standard (ml) / 50* = serial dilution

* Divide by 50 as all dilutions were made up to 50 ml.

Analytical grade methanol was used to make up the 50ml.

Appendix 2.5 The Persist Model (Walker 1974)

To run this program you must have a file containing daily values of maximum and minimum air temperatures and daily rainfall (mm)

MaxTemp MinTemp Rain

1. *What is the name of your input weather data file?*

2. What is the title to describe your printed output?

3. How many days of input weather data are there in your input data file?

4. What is the interval (days) for the printed output?

   This can be any number, but either 2, 5 or 10 is often the most useful.

5. What is the altitude of your site (metres)?

6. What is the latitude of your site (degrees)?

   You can use your weather data file to make several different model runs e.g. with different herbicides, different soils or different application times.

7. How many different runs of the model will you make?

8. What is the subheading for this run of the model?

9. What is the field capacity (% w/w) of your soil?

10. What is the bulk density of the soil (g/cubic cm)?

11. What is the initial amount of herbicide? This can be specified as kg/ha, mg/kg or as a percentage (i.e. 100). The output will then be in these units.

12. The next input data are constants to characterise the temperature and moisture dependence of degradation.

    An estimate of the half-life at a known moisture and temperature is the minimum requirement.

13. Specify the 1st and last days for the simulation run relative to the total number of days in your weather data file.

Month	March		April		May		June		July	
Days	Maxtemp	Mintemp	Mintemp	Maxtemp	Mintemp	Maxtemp	Mintemp	Maxtemp	Mintemp	Maxtemp
1	8.9	-1.1	10.3	6.7	13.5	4.5	20.1	5.2	18.6	8.9
2	12.4	3.3	11.1	6.7	15.9	3.3	14.3	10.8	17.4	9.1
3	13.6	7.4	11.3	5.5	13.4	6.8	16	10	18.9	10.7
4	9	3.5	12.4	6.4	15.1	8	17.2	7.5	20	10.4
5	11.3	2.7	11.1	7.2	12.3	5.5	16.6	9	20.2	12
6	12.3	3.7	13.5	3.4	14.9	9.3	20	11	18.5	13.1
7	11.6	8.7	11.7	-0.1	15	10.9	18.1	9.8	18.2	11.9
8	7.6	5.4	10.5	3.8	19	11.4	17.2	5.7	19.7	9
9	9.5	-1.6	7.8	5.3	18.6	12.4	19.2	12.3	18.8	13.8
10	10.2	1.3	3.6	0.6	14.5	4.3	18.2	9.5	20.3	9.6
11	8.6	4.5	7.5	0.2	11.9	8.6	13.2	6.1	17	9.9
12	8.6	-0.3	7.3	-2.9	16.5	8.9	15.9	4.8	19.1	13.8
13	9	2.8	6.4	-2.5	21.1	10.1	14.7	8.7	16.7	9.5
14	10.8	7	7.6	-1	20	10.8	15.7	8.8	16.6	10.4
15	10.8	8	6.9	0	22.9	12.3	14.2	10.6	19	10.6
16	10.4	7	8.1	-0.4	22.2	9.8	16.8	10.3	21	8.4
17	12.5	706	9.4	3.4	20	4.8	16.7	6.2	19	10.5
18	12.5	2.5	11.4	1.9	21.6	5.9	18.5	10.9	19.1	9.3
19	11	1.3	9.4	-0.5	22.4	6.6	23.6	14.1	19	11.2
20	10.9	2.2	12	3.2	22.3	6.9	24.6	15.2	21.9	14.4
21	10.8	6.8	11	-0.4	17.1	10.6	20.5	15.4	19.9	11
22	11.8	-1.5	15	8.2	18.4	8.7	18.7	11.6	19.5	12.1
23	8.7	-1	11.8	7.7	14.1	7.5	19.5	13.1	18.4	13.8
24	8.2	4.7	16.5	4.3	16.9	10.3	18.9	15	18.2	7
25	10.6	5	14.9	7.5	16.9	10.6	18.1	8.1	20.1	5.5
26	13.4	8.2	12.7	7.3	12.4	8.8	16.2	10.9	18.5	11.2
27	14.6	6.8	14.3	-0.2	12.8	2.3	17.7	11	21.4	7.9
28	16.7	8.7	14.2	1.8	15.8	4.9	19	9.2	21.3	13.8
29	13.8	7.9	15.5	3.6	19.5	8.4	16.7	11.8	19	14.2
30	15.2	11.2	12.8	7.1	20	3.2	15.9	7.5	17.9	12.1
31	15	3.5			19.3	10.3			17.5	13.1

Appendix 2.5 a The maximum and minimum air temperatures measured at Shawbury weather station from 09 this day - 09 next day

Day	Month				
	March	April	May	June	July
1	1.4	1.4	0.0	11.4	0.1
2	7.2	7.4	0.2	7.6	0.1
3	16.8	7.4	0.1	1.4	0.1
4	1.6	4.2	0.1	0.0	0.0
5	7.4	1.0	0.4	4.2	0.2
6	5.4	0.0	0.4	0.4	0.1
7	0.8	3.5	0.1	1.8	0.2
8	0.0	2.4	1.6	6.8	0.2
9	0.0	11.4	0.0	0.4	0.1
10	3.8	1.6	2.4	7.2	1.2
11	0.1	0.1	0.1	0.0	4.6
12	2.6	0.5	0.1	4.0	1.8
13	0.1	7.8	0.2	1.6	3.8
14	0.0	0.1	0.1	0.2	0.2
15	0.0	0.1	0.2	0.4	0.1
16	0.0	0.1	0.0	1.2	0.1
17	0.0	0.0	0.0	6.4	0.2
18	0.0	7.4	0.0	0.6	0.8
19	0.0	5.8	0.0	0.0	0.8
20	0.0	1.0	0.0	0.6	7.6
21	0.0	5.8	0.0	0.1	1.0
22	0.0	2.4	0.1	0.6	3.2
23	1.6	2.2	0.6	0.6	0.6
24	11.8	0.6	0.1	1.4	0.2
25	1.4	5.4	2.4	2.4	0.1
26	0.1	0.2	2.4	7.8	2.4
27	0.1	0.4	0.1	3.4	0.1
28	0.1	0.1	0.8	0.2	0.1
29	2.6	0.0	0.0	0.1	1.2
30	0.0		1.2	0.1	4.6
31	0.0		0.0		0.5

Appendix 2.5b Rainfall (mm) measured at Shawbury weather station from 09 this day - 09 hrs next day

Appendix 2.5 cont.

Table.a. *Dry bulk density of the soil from each site*

Site	wht. bag and wet soil	wht. bag and dry soil	oven dry mass	height of soil (cm)	volume of soil (cm^3)*	dry bulk density (g /cm^3)
Bigwood	262.04	221.5	249.84	5	251.33	0.99
Heath	259.72	230.27	249.88	4.3	216.14	1.16
Davies	261.96	230	249.87	4.8	241.27	1.04
Bubb	262.12	237.86	249.90	4.7	236.25	1.06
Maddocks	262	224.49	249.85	5.2	261.38	0.96
WJ	261.9	221.45	249.84	5.4	271.43	0.92
Front	261.94	223.92	249.85	4.7	236.25	1.06
Crab	262.02	229.77	249.87	4.5	226.19	1.10
Marsh	259.74	225.66	249.86	5	251.33	0.99
Otter	261.9	229.5	249.87	5	251.33	0.99

* $\pi r^2 = 50.27$

Table.b. *Available water capacity of the soil at each site*

Site	wht. bag and wet soil	wht. bag and dry soil	% by mass	dry bulk density (g /cm^3)	AWC	70 % AWC
Bigwood	327.58	254.6	29.19	0.99	29.02	20.31
Heath	328.77	254.35	29.77	1.16	34.42	24.09
Davies	329.33	258.5	28.33	1.04	29.34	20.54
Bubb	335.56	260.82	29.90	1.06	31.62	22.14
Maddocks	328.25	258.59	27.86	0.96	26.63	18.64
WJ	342.47	245.08	38.96	0.92	35.86	25.10
Front	337.41	253.01	33.76	1.06	35.70	24.99
Crab	333.47	251.9	32.63	1.10	36.04	25.23
Marsh	331.63	256.5	30.05	0.99	29.88	20.91
Otter	338.8	259.19	31.84	0.99	31.66	22.16

Appendix 2.6~~A1~~ - Exponential Regression Analysis

Bigwood	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	9115.92	84.3	148.98	< .001
+ separate *a* parameters	1	1335.65	90.7	21.83	< .001
+ separate *b* parameters	1	32.33	90.4	0.53	0.476
+ separate *d* parameters	1	533.7	93.1	8.72	0.008
residual	19	61.19			

Stockton	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	9409.47	67.7	243.58	< .001
+ separate *a* parameters	1	6321.32	92.8	163.64	< .001
+ separate *b* parameters	1	0.49	92.5	0.01	0.911
+ separate *d* parameters	1	874.06	96.1	22.63	< .001
residual	22	38.63			

Heaths	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	8135.79	65.4	112.75	< .001
+ separate *a* parameters	1	4698.4	86	65.11	< .001
+ separate *b* parameters	1	53.8	85.6	0.75	0.397
+ separate *d* parameters	1	1346.3	91.9	18.66	< .001
residual	22	72.2			

Round	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	10570.31	71.4	446.46	< .001
+ separate *a* parameters	1	5309.68	90.9	224.27	< .001
+ separate *b* parameters	1	11.98	90.6	0.51	0.484
+ separate *d* parameters	1	1782.22	97.8	75.28	< .001
residual	22	23.68			

Tables *a-d* Results from exponential (or sympotic regression) for experimental data and modelled data at each site

Appendix 2.6~~A85~~

Common	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	11919	91.2	147.51	< .001
+ separate *a* parameters	1	330.4	92.2	4.09	0.055
+ separate *b* parameters	1	4.19	91.9	0.05	0.822
+ separate *d* parameters	1	9.63	92.2	0.12	0.733
residual	22	80.8			

Search	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	10941.2	94.4	199.67	< .001
+ separate *a* parameters	1	0.32	94.2	0.01	0.940
+ separate *b* parameters	1	36.67	93.8	0.67	0.423
+ separate *d* parameters	1	1.61	94.1	0.03	0.866
residual	21	54.8			

Front	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	12864.94	94.6	241	< .001
+ separate *a* parameters	1	9.42	94.4	0.18	0.678
+ separate *b* parameters	1	18.55	94.3	0.35	0.562
+ separate d parameters	1	145.5	94.7	2.73	0.113
residual	22	53.38			

Crab	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	12310	92.4	234.9	< .001
+ separate *a* parameters	1	514.2	94.3	9.81	0.006
+ separate *b* parameters	1	89.8	94.4	1.71	0.207
+ separate *d* parameters	1	276	95.4	5.27	0.064
residual	18	52.4			

Tables *e-h* Results from exponential (or sympotic regression) for experimental data and modelled data at each site

Appendix 2.6

Malt	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	11394.4	79.8	318.49	< .001
+ separate *a* parameters	1	3541.83	93.2	99	< .001
+ separate *b* parameters	1	566.2	95.3	15.83	< .001
+ separate *d* parameters	1	381.96	96.8	10.68	0.004
residual	20	35.78			

Otter	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	8321.19	72.3	136.56	< .001
+ separate *a* parameters	1	3727.3	89.9	61.17	< .001
+ separate *b* parameters	1	137.73	90.1	2.26	0.147
+ separate *d* parameters	1	539.86	92.7	8.86	0.007
residual	22	60.93			

Tables *i -j* Results from exponential (or sympotic regression) for experimental data and modelled data at each site

Appendix 3.1 Nematode population density assessments

Calculations for the number of juveniles per gram of root

Number of eggs g^{-1} = no. of cysts x (no. of eggs in 1 ml x 50 /no. of crushed cysts) / 200

Appendix 4.1 Calculations for the available water content of a soil

AWC = (θg x 100 = % by mass) x dry bulk density = % by volume.

$\theta g = \frac{\text{mass of water}}{\text{mass of dry soil (g)}}$

Seventy percent of the AWC was then calculated.

Appendix 4.2
A preliminary assessment of the techniques used to obtain the AWC of a soil.

Three soils from the same site were analysed using the methods described in Chapter 4, section 4.2.2. g.

Dry bulk density = 0.7 g / cm^3
AWC = 100*(wet – dry / dry) * dry bulk density

100 * (113.23 - 89.72 / 104.59)* 0.7 = 15.73	Average = 15.75
100 * (112.16 - 89.30 / 102.26)* 0.7 = 15.58	Standard deviation = 0.111
100 * (112.34 - 89.03 / 102.79)* 0.7 = 15.87	Standard error = 0.06

Results were significantly different, however this was with weights of soils taken to two decimal places. The fact that with 3 replicates the data was within 0.5 g range showed that results were replicable.

Appendix 4.3 Initial concentrations of oxamyl applied to the incubated soils

An initial concentration of 2.62 μg/g was decided because growers apply oxamyl at a full rate of 5.5 kg ha^{-1} ai.

Recommended depth of incorporation of Vydate = 15 cm
Incorporated volume of soil = 10,000 x 0.15 = 1500 m^3 (1 ha = 10,000 m^2)

5500g of oxamyl in 1500 m^3 soil = 3.66 g oxamyl / m^3 (1,000,000 cm^3)

Average dry bulk density from the 10 sites = 1.4 g/cm^3

3.66g Vydate ai = 1.4 x 10^6 cm^3 = 1,400,000 g of soil = 2.619 μg / g soil of oxamyl

Appendix 4.4 - Exponential Regression Analysis

Bigwood	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	4772.60	88.1	188.40	< .001
+ separate a parameters	1	614.80	94.6	24.27	0.003
+ separate b parameters	1	38.29	94.4	1.51	0.265
+ separate d parameters	1	225.52	97.4	8.90	0.025
residual	6	25.33			

Stockton	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	3953.93	66.9	133.45	< .001
+ separate a parameters	1	2236.42	91.1	75.48	< .001
+ separate b parameters	1	508.78	97.0	17.17	0.006
+ separate d parameters	1	11.50	97.3	0.39	0.556
residual	6	29.63			

Heaths	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	3688.03	77.9	92.35	< .001
+ separate a parameters	1	1098.64	91.9	27.51	0.002
+ separate b parameters	1	286.75	95.1	7.18	0.037
+ separate d parameters	1	1.17	95.8	0.03	0.870
residual	6	39.94			

Round	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	3870.76	65.1	70.02	< .001
+ separate a parameters	1	2238.33	89.2	40.49	< .001
+ separate b parameters	1	502.90	94.9	9.10	0.024
+ separate d parameters	1	19.98	94.4	0.36	0.570
residual	6	55.28			

Appendix 4.4*(a -j)* Results from exponential (or sympotic) regression with groups or rectangular (hyperbola) for experimental and laboratory data

Appendix 4.4

Common	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	5041.5	89.1	46.82	< .001
+ separate a parameters	1	248.6	89.3	2.31	0.179
+ separate b parameters	1	65.9	90.8	0.61	0.464
+ separate d parameters	1	29.0	90.4	0.27	0.622
residual	6	107.7			

Searchlight	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	5456.91	96.8	147.68	< .001
+ separate a parameters	1	16.47	96.6	0.45	0.529
+ separate b parameters	1	2.14	96.2	0.06	0.818
+ separate d parameters	1	50.9	96.4	1.38	0.285
residual	6	221.71			

Front	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	4218.42	92.2	66.93	< .001
+ separate a parameters	1	28.15	92.8	0.45	0.529
+ separate b parameters	1	41.07	93.1	0.65	0.450
+ separate d parameters	1	27.72	93.5	0.44	0.532
residual	6	63.03			

Crab	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	7116.24	94.2	79.3	< .001
+ separate a parameters	1	125.19	94.7	1.4	0.282
+ separate b parameters	1	21.01	94.1	0.23	0.646
+ separate d parameters	1	20.95	94.7	0.23	0.646
residual	6	89.74			

Appendix 4.4

Marsh	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	5852.03	74.8	110.64	< .001
+ separate a parameters	1	1735.11	87.8	32.80	0.001
+ separate b parameters	1	938.55	96.0	17.74	0.006
+ separate d parameters	1	54.51	96.1	1.03	0.349
residual	6	52.89			

Otter	DF	Mean Square	cumulative % variation accounted for	F - value	p -level
all parameters constant	2	3390.53	71.3	71.08	< .001
+ separate a parameters	1	1473.27	90.6	30.88	0.001
+ separate b parameters	1	228.57	93.4	4.79	0.015
+ separate d parameters	1	88.19	94.1	1.85	0.223
residual	6	47.7			

Appendix 5.1

Parameters	DF	Mean Square	Cumulative % variation accounted for	F - value	p - level
all parameters constant	1	9937.32	76.4	125.33	<.001
+ separate a parameters	1	1825.33	91.4	23.02	0.001
+ separate b parameters	1	254.03	93.1	3.20	0.111
residual	8	79.29			

Table 5.1a *Comparing the decline of aldicarb sulphoxide in aldicarb treated and untreated soils*

Parameters	DF	Mean Square	Cumulative % variation accounted for	F - value	p - level
all parameters constant	1	10105	82.1	80.25	<.001
+ separate a parameters	1	918.8	89.4	7.3	0.027
+ separate b parameters	1	39.8	88.5	0.32	0.589
residual	8	125.9			

Table 5.1b *Comparing the decline of aldicarb sulphoxide in aldicarb treated and untreated soils*

Table 5.1 *Linear regression analysis with groups of the degradation of aldicarb sulphoxide in treated and untreated soils*

Appendix 5.2

Parameters	DF	Mean Square	Cumulative % variation accounted for	F - value	p - level
all parameters constant	2	4501.06	51.9	65.94	<.001
+ separate *a* parameters	1	3780.39	80.8	55.38	<.001
+ separate *b* parameters	1	1592.56	94.9	23.33	0.002
residual	7	68.26			

Table 5.2a *Comparing the decline of total aldicarb in aldicarb treated and untreated soils*

Parameters	DF	Mean Square	Cumulative % variation accounted for	F - value	p - level
all parameters constant	2	4705.45	92	68.73	<.001
				0.01	0.923
+ separate *a* parameters	1	0.69	91	0.53	0.494
+ separate *b* parameters	1	36.2	90.3	3.07	0.131
+ separate *d* parameters	1	209.83	92.5		
residual	6	68.46			

Table 5.2b *Comparing the decline of total aldicarb in oxamyl treated and untreated soils*

Table 5.2 *Linear regression analysis with groups or exponential (or sympotic regression) for treated and untreated soils*

Appendix 5.3

Parameters	DF	Mean Square	Cumulative % variation accounted for	F - value	p - level
all parameters constant	2	8317.44	93.6	55.78	<.001
+ separate *a* parameters	1	7.89	90.7	0.11	0.756
+ separate *b* parameters	1	0.72	91.9	0.01	0.925
+ separate *d* parameters	1	6.79	92.9	0.09	0.773
residual	6	447.36			

Table 5.3a *Comparing the decline of oxamyl in aldicarb treated and untreated soils*

Parameters	DF	Mean Square	Cumulative % variation accounted for	F - value	p - level
all parameters constant	2	5892.7	94.2	138.95	<.001
+ separate *a* parameters	1	313.69	96.2	7.4	0.035
+ separate *b* parameters	1	11.38	96.6	0.27	0.623
+ separate *d* parameters	1	11.17	96.9	0.26	0.626
residual	6	42.41			

Table 5.3b *Comparing the decline of oxamyl in oxamyl treated and untreated soils*

Tables 5.3 *Results from exponential (or sympotic regression) for treated and untreated data*